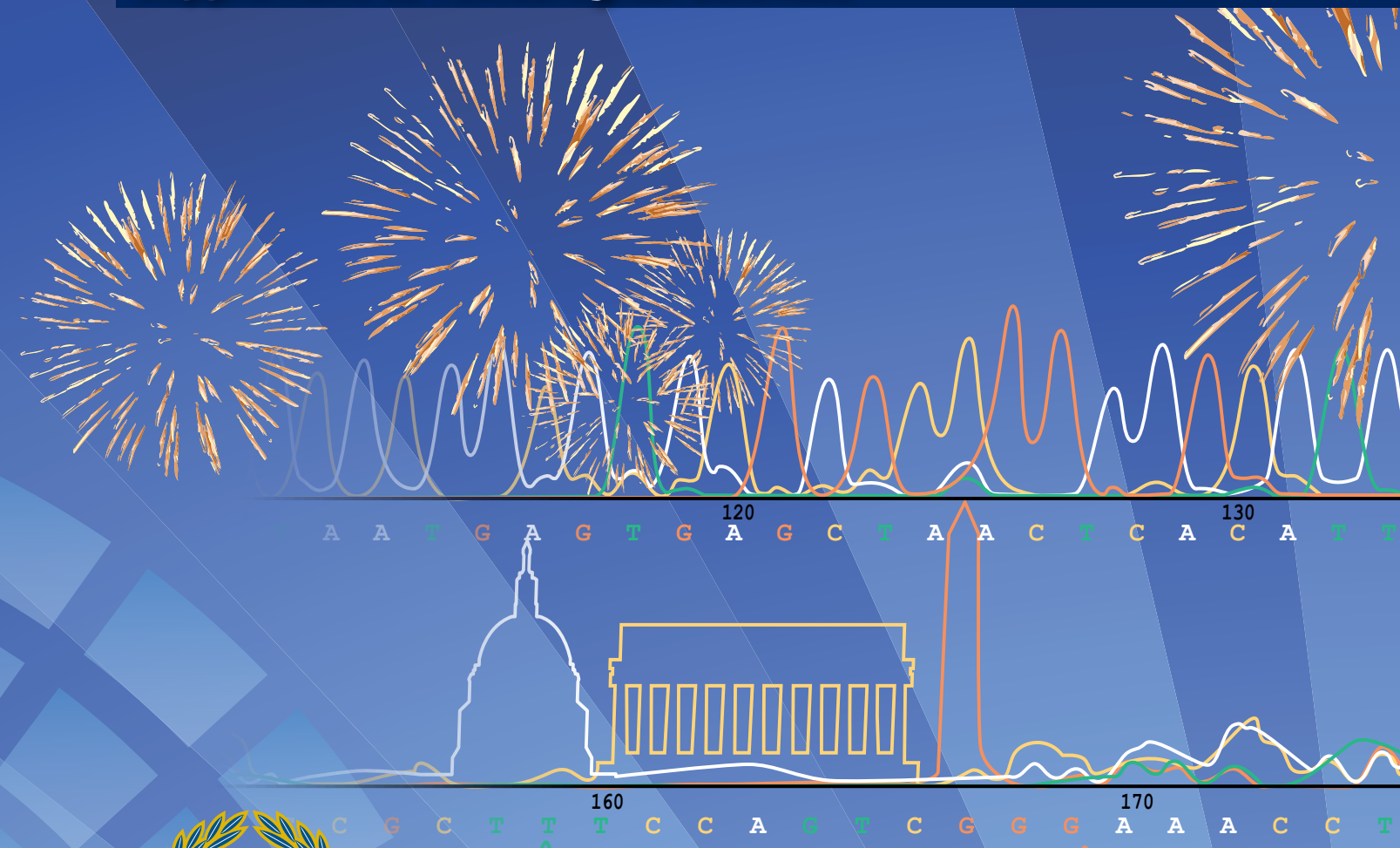
The Toxicologist

Supplement to *Toxicological Sciences*



Celebrating 50 Years of Service in Science

Anniversary Annual Meeting and ToxExpo™ Washington, D.C.

An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

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

www.toxicology.org

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Deadline for Proposals for SOT 2012 Annual Meeting Sessions: April 30, 2011

WHY SUBMIT A PROPOSAL?

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

2012 Thematic Approach

The Scientific Program Committee will continue the thematic approach for the 2012 Annual Meeting. Additional details regarding the themes will be available on the SOT Web site.

Please note that while we are actively soliciting proposals for the themes, all proposal submissions will be reviewed for their timeliness and relevance to the field of 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

Submit your proposal on-line at www.toxicology.org

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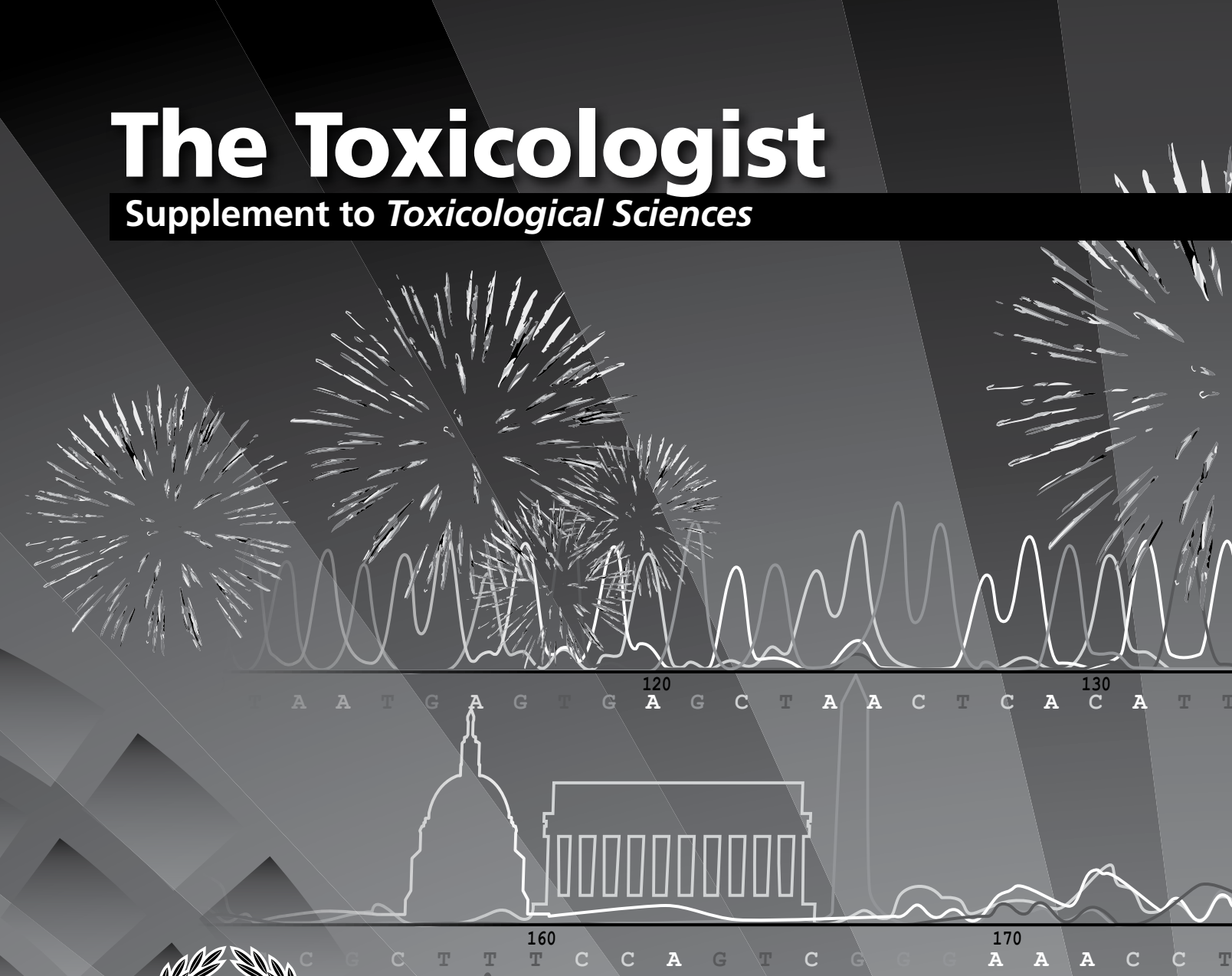

SOCIETY OF TOXICOLOGY
50th Anniversary
1961–2011

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

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CE 1 BIODEGRADABLE MATERIALS FOR TISSUE ENGINEERING: APPLICATIONS AND SAFETY ASSESSMENT.

R. P. Brown[1] and R. W. Hutchinson[2]. [1]*U.S. FDA, Silver Spring, MD and* [2]*Johnson & Johnson, The Woodlands, TX.*

The incorporation of biodegradable materials as a fundamental component in tissue regeneration strategies began in the early 1980's and continues today. The function of a biodegradable material is to act as a temporary support matrix for transplanted or host cells so as to restore, maintain, or improve tissue. In order for this function to be achieved, biodegradable materials must undergo a number of critical examinations to define their properties. For example, degradation rate, degradation products, and the tissue response to these products must all be characterized. In this presentation we will introduce a number of natural and synthetic biodegradable materials that are commonly considered in regenerative medicine, as well as some recently developed novel materials. The techniques utilized to describe their physical properties and the relationship between physical properties and tissue response will be examined, and advanced techniques for material characterization and toxicological effects will be considered. Finally, the application of these biodegradable materials in tissue engineering strategies will be described.

CE 2 BEST PRACTICES FOR DEVELOPING, CHARACTERIZING, AND APPLYING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS IN RISK ASSESSMENT.

B. Meek[1] and J. C. Lipscomb[2]. [1]*University of Ottowa, Ottowa, ON, Canada and* [2]*U.S. EPA, Cincinnatti, OH.*

This course is aimed at increasing confidence in the evaluation and application of PBPK models in quantitative health risk assessments, through systematic consideration of relevant criteria for their development and documentation, based on guidance. These principles (Best Practices for PBPK Modeling Applied to Health Risk Assessment) have been recently collected and expanded upon in guidance published by the WHO International Programme on Chemical Safety (2010), and have been the subject of several other peer-reviewed publications. The course comprises lectures describing the link between mode of action, dose-response characterization and risk assessment, and the role of PBPK models in reducing and characterizing uncertainty and variability. The course will present principles for the development, characterization, and communication and criteria for evaluation of PBPK models for risk assessment applications. A novel inclusion will be a projected demonstration of real-time changes in model outcome that depend on choice of model parameter values (e.g., breathing rate, metabolic activity). The demonstration of user-friendly model development software will be demonstrated in the final lecture. This will show the impact of choices for parameter values, and models will be exercised and the results interpreted to produce quantitative values to be used in place of uncertainty factors in health risk assessments.

CE 3 CURRENT NON-CLINICAL STRATEGIES AND METHODS FOR EVALUATING DRUG-INDUCED CARDIOVASCULAR TOXICITY.

H. Wang[1] and D. J. Murphy[2]. [1]*Genentech Inc., South San Francisco, CA and* [2]*GlaxoSmithKline Pharmaceuticals, King of Prussia, PA.*

Cardiovascular (CV) toxicity is among the major causes of withdrawal of drugs or restriction in their labeling and has had an impact on public health and the rising cost of developing new drugs. Early identification and characterization of CV liabilities, better understanding of the predictive values of nonclinical models, and an integrated and iterative approach during drug development could greatly facilitate the development of safe and effective medicines for patients. This course will describe the current *in vitro* and *in vivo* methods for evaluation of functional and structural CV liabilities, and discuss the strategies that can be applied at early stages of drug development to help reduce attrition and to avoid unanticipated liabilities at later development stages in either animal studies or in the clinic. Study design and data interpretation will be discussed, as well as the advantages, limitations, and future directions of current methods involving both functional and structural assessments. Specific topics such as integration of functional CV endpoints into repeat-dose toxicity studies, methods for identification and characterization of cardiac arrhythmia, and special considerations for testing oncology and diabetes drugs and biologics will be covered. In addition, case study examples will be provided to highlight how these data can be used to inform decisions at different stages of development. A regulatory perspective on the challenges and gaps of CV safety evaluations and opportunities available to improve the overall CV safety assessment paradigm will also be presented. Overall, this course will provide participants with a broad overview of the types of drug-induced CV liabilities, the current nonclinical strategies and methodologies for early detection of CV liabilities, and a regulatory perspective on the impact of CV toxicity on the drug-development process.

CE 4 DEALING WITH THE DATA DELUGE: A LIVE DATA DISCOVERY AND ANALYSIS COURSE.

M. E. Gillespie[1] and S. M. Bello[2]. [1]*Saint Johns University, Jamaica, NY and* [2]*Jackson Laboratory, Bar Harbor, ME.*

Using web-based resources and tools to gain novel scientific insights and advance your research is a significant step for all researchers. As the pace of science accelerates, experimental technologies continue to evolve and the quantity of data increases. With the evolution in biological research comes an increasing reliance on database resources and computational analysis tools to parse and integrate this growing mass of biological data. The field of toxicology is not exempt from these challenges. In this course, a diverse group of data resources have joined their efforts to present a unique series of hands-on tutorials. The tutorials follow a hypothetical researcher through the various stages of experimental design and data analysis, demonstrating how the different workshop resources can be used to facilitate all steps of the research process. Participants will identify orthologous biological information across different species; identify biological trends (pathway, function, phenotype, xenobiotic interactions) within a submitted data set; investigate an individual data set with on-line resources, identifying supplementary information available across multiple data sets; and gain hands on experience with formatting and submitting data to a diverse set of on-line data resources.
Today toxicologists must select appropriate model organisms, manage abundant high-throughput data, understand legacy data, and develop pathway-based understanding of environmental factors influencing biological systems. Mastery of these concepts improves toxicity prediction while providing insights into environmentally influenced diseases and phenotypes. A clear understanding of the diverse online data resource aims and limitations equips the researcher with the best combination of resources to effectively address their questions.

CE 5 EPIGENETICS IN TOXICOLOGY: INTRODUCTION, MECHANISTIC UNDERSTANDING, AND APPLICATIONS IN SAFETY ASSESSMENT.

M. S. Mitra[1] and T. Sussan[2]. [1]*Washington University School of Medicine, Saint Louis, MO and* [2]*Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.*

Epigenetics refers to molecular mechanisms that cause heritable changes in gene expression without altering the DNA sequence. The most widely studied epigenetic mechanisms encompass DNA methylation, histone modifications, and gene regulation by non-coding RNAs, such as microRNAs. Typically these mechanisms are required for normal cellular development and differentiation; however, perturbations in them can lead to diseases, notably cancer. Increasing evidence suggests that environmental factors such as diet, stress, and exposure to radiation and xenobiotics can induce heritable changes in the epigenetic status, potentially affecting the health of the present and future generations. Importantly, the long-term and life-threatening consequences of environment/chemical-induced changes in epigenetics makes this field a critical area for future exploration by toxicologists. The course will begin by introducing the fundamental concepts of epigenetics and reviewing the various underlying mechanisms. Methods to assess epigenetic changes will be discussed, followed by a discussion of the role of DNA cytosine methylation in the regulation of carcinogen-inducible CYP450 genes. Mechanistic understanding of the role of microRNAs in the regulation of cellular toxicity and the influence of environment on epigenetics that cause developmental defects will also be presented. Finally, the future of epigenetics in toxicology and its potential applications for safety assessment will be discussed. Students as well as toxicologists working in academia, federal and pharmaceutical industries, and researchers interested in mechanistic toxicology will benefit from taking this course.

CE 6 PROTECTING HUMAN HEALTH: USE OF TOXICOLOGICAL AND EPIDEMIOLOGICAL DATA IN DETERMINING SAFE LEVELS FOR HUMAN EXPOSURE.

E. P. Hayes[1] and T. Gordon[2]. [1]*EP Hayes Toxicology Services LLC, Longmont, CO and* [2]*New York University School of Medicine, Tuxedo, NY.*

Toxicological and epidemiological data are essential to risk assessment processes used to determine acceptable levels of exposure, both for the general public who may be exposed to pollutants *via* ambient air and/or drinking water, for workers

who handle chemicals, and for patients exposed to both active pharmaceutical ingredients (API) and impurities in the product. The goal of this course is to provide an understanding of the regulatory background and the practical application of both toxicological and epidemiological information in setting exposure levels considered to be protective of public health. The objectives of this course are 1) to describe the regulatory requirements that underlie development of acceptable levels of exposure for either the general population or select populations (workers, patients) *via* the media described above; and 2) to describe the evaluation of toxicological and epidemiological data in determining acceptable levels of exposure. Case studies of representative compounds will illustrate the processes. National and international agencies (e.g., U.S. Environmental Protection Agency, U.S. Occupational Health and Safety Administration, U.S. Food and Drug Administration, and European Medicines Agency), as well as non-profit organizations (e.g., American Conference of Government Industrial Hygienists, International Committee on Harmonization) and employers, have well-defined processes for establishing ambient air quality standards, drinking water maximum contaminant, permissible exposure limits for the workplace and acceptable identification, reporting, and safety thresholds for impurities in drug products. The course will highlight legal and customary definitions of "acceptable risk," as well as risk assessment methods for evaluating data to estimate risk levels under these programs. The regulations and/or guidances will be detailed and approaches used to comply with them will be described.

CE 7 DRUG HYPERSENSITIVITY REACTIONS: RISK ASSESSMENT AND MANAGEMENT.

M. Popovic[1] and J. Whritenour[2]. [1]*Eli Lilly & Company, Indianapolis, IN and* [2]*Pfizer Global Research and Development, Groton, CT.*

Drug hypersensitivity reactions are not a common problem in drug development; however, when they do occur they can have a significant impact on the drug candidate's developmental success. Drug hypersensitivity reactions are usually discovered in Phase II or III clinical trials, or in the post-marketing phase. Once allergic reactions are observed in patients, one needs to determine if the reaction is mediated by an immune response to the drug, or another mechanism. There are a few *ex vivo* diagnostic methods that can be used to identify immune-mediated reactions, but one needs to be aware of the limitations and advantages of each approach. *In vitro* methods, or animal models presently being developed to predict drug's potential to trigger hypersensitivity reaction in the patient population are being developed, but at present, they have significant limitations. Risk management strategies may include selection of patient populations based on the HLA haplotype. This course is intended as an introduction for those with limited background in the area of hypersensitivity, or allergic reaction to drugs. The focus of the course will be on systemic hypersensitivity reactions (drug administered orally or parenterally) and will include discussions both on drugs that are small molecules and biologics.

CE 8 TOXICOLOGY AND RISK ASSESSMENT OF CHEMICAL MIXTURES.

J. Simmons[1] and C. J. Borgert[2]. [1]*U.S. EPA, Research Triangle Park, NC and* [2]*Applied Pharmacology Toxicology, Inc., Gainesville, FL.*

Assessment of the safety and risk of environmental chemicals, pharmaceuticals, consumer and personal care products, pesticides, and food additives increasingly requires consideration of the potential pharmacological and toxicological interactions that might occur as these agents are encountered as mixtures by patients, consumers, and through environmental exposures (e.g., mixtures present in air, water, soil). Both toxicological evaluations and risk assessments of mixtures of chemicals are complex due to the potential pharmacokinetic and pharmacodynamic mechanisms that might result in nonadditive interactions. While greater than expected toxicity is of most concern for environmental exposures, both less than and greater than additive toxicity are of pharmacological concern. Toxicological evaluation of chemical mixtures necessitates study designs, methods of analysis, and limits on interpretation not required for single chemicals. This course will cover the fundamentals of study design and data analysis for mixtures that apply to all classes and categories of chemicals encountered by humans and animals, regardless of market application. The objectives of this course are to 1) describe the basic principles that underlie modern concepts of the toxicology and risk assessment of chemical mixtures; 2) survey the basic tools and techniques needed to design, conduct, analyze and interpret experimental data with defined or complex mixtures of chemicals; and 3) review the guidance, underlying assumptions, and techniques used in risk assessment of chemical mixtures. This course will be of interest to experimentalists who wish to conduct studies on mixtures that are meaningful for evaluation of risk as well as safety and risk assessors who must evaluate and apply data on mixtures and interactions in assessments.

CE 9 APPLICATIONS OF COMPUTATIONAL SYSTEMS BIOLOGY FOR TOXICOLOGY.

M. E. Andersen[1] and R. B. Conolly[2]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [2]*U.S. EPA, Research Triangle Park, NC.*

The field of toxicity testing and risk assessment is undergoing a shift from reliance on high-dose animal studies towards increased use of human *in vitro* systems that promise to provide mechanistic understanding of toxicity for environmentally relevant low-dose exposure. For this fundamental change, toxicologists will need to adopt more integrated experimental and computational approaches to resolve the structures of key signaling pathways, which are composed of functional network motifs, and to understand the consequences of chemical perturbation on the dynamic and steady-state behaviors of these pathways. This course introduces state-of-the-art computational systems biology tools that are being used for organizing and understanding molecular circuits under both physiological and perturbed conditions. A broad overview will first provide a historical context of dose-response studies based on understanding mode of action through cellular pathway perturbation. The course will describe signaling properties of a suite of recurring network motifs, including ultrasensitivity, feedback, and feedforward loops, to appreciate the basic building blocks of complex biochemical pathways and networks. Secondly, focusing on the DNA damage response and cell cycle progression pathways, we will illustrate how these network motifs are organized into molecular circuits to give rise to higher-level cellular functions and if perturbed, how functional aberrations result. Signal transduction networks activated by growth factors are then examined to show how pathway cross-talk and feedback loops define the activation logic of the downstream MAPK, which is a key determinant of cell growth and survival. Finally, we will shows how stochastic gene expression and the resulting non-genetic cell-to-cell variability plays a role in influencing dose response curves using examples such as B cell differentiation and its disruption by dioxin. The course concludes with a short summary and suggestions for applying these computational systems biology tools to future toxicity testing.

CE 10 EVALUATING TOXICITY OF ENGINEERED NANOMATERIALS: ISSUES WITH CONVENTIONAL TOXICOLOGY APPROACHES.

S. S. Nadadur[1] and F. A. Witzmann[2]. [1]*NIEHS-DERT, Research Triangle Park, NC and* [2]*Indiana University School of Medicine, Indianapolis, IN.*

Engineered nanomaterials (ENMs) have become an integral part of numerous consumer products, cosmetics, building materials, medical devices, therapeutic agents, and environmental remediation. Global demand for nanomaterials and nano-enabled devices has been projected to surpass $3.1 trillion by 2015. The widespread use of nanotechnology-derived products presents opportunities for intentional and unintentional exposure to ENMs. The size and size-dependent novel physical and chemical properties that make ENMs unique compared to micro-scale products of similar chemical composition makes it difficult to determine their interaction with biological matrices. The recent flood of toxicology literature without proper physical and chemical characterization of ENMs proposes adverse to no health effects for certain common ENMs such as carbon nanotubes and metal oxide nanoparticles. The course will provide an overview of the issues facing nanotechnology that the scientific community must grapple with in regard to predicting toxicity and biological outcomes associated with nanoscale properties and the need to identify and integrate novel approaches for safety of ENMs. To begin, focus will be placed on the importance of incorporating physical and chemical characteristics of ENMs in interpreting biological data. Then, *in vitro* approaches using multiple parameters to classify ENMs toxicity profile will be covered. Altered proteomic profiles in a model *in vitro* system to understand molecular alterations will be explored. Finally, the interpretation of data from *in vivo* studies using inhalational routes of exposure will be discussed. The goal of this course is to encourage both the novice and the toxicologist trained in conventional toxicity assessment to think outside the box to design rational toxicology studies in evaluating the safety of ENMs that are currently in use, and to develop models to predict potential toxicity of second and third generation ENMs.

CE 11 NEW TECHNOLOGIES AND APPROACHES IN GENETIC TOXICOLOGY AND THEIR EXPANDING ROLE IN GENERAL TOXICOLOGY AND SAFETY ASSESSMENT.

J. C. Bemis[1] and J. C. Sasaki[2]. [1]*Litron Laboratories, Rochester, NY and* [2]*Johnson & Johnson, Raritan, NJ.*

For decades, genetic toxicology and the "genetox battery" have been a well-established part of safety testing for pharmaceuticals and other chemical agents. Recent advances in experimental methodologies are contributing to a change in the way

that genetic toxicology data are generated and incorporated in the disciplines of toxicology and safety testing. The intention of this course is to illustrate the broader impact that new genetic toxicology approaches are having on drug/chemicals safety assessment and human risk analysis. The structure of the course will provide examples of (1) Early discovery/high-throughput genotoxicity screening of chemical entities; (2) Integration of genetic toxicology assays with repeat-dose *in vivo* toxicology studies; and (3) New approaches for genotoxicity risk assessment, and conclude with an update on genotoxic impurity management strategies for pharmaceuticals. Speaker presentations will illustrate how genotoxicity testing is evolving from a hazard identification based-discipline to an integrated approach that may ultimately yield quantitative information that can be used for human risk assessment.
This course should be of interest to experienced genetic toxicologists as well as those involved in general toxicology who want to learn about how incorporation of new genotoxicity methods can improve test predictivity, lower costs, reduce animal use, and may ultimately be applied to human risk assessment

CE 12 PRACTICAL HOW-TO AND PITFALLS ASSOCIATED WITH CURRENT EPIGENETIC STUDIES.

R. Rasoulpour[1] and C. Qin[2]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Merck & Co., Inc., West Point, PA.*

The study of toxicant-induced epigenetic modifications is greatly expanding in complexity and scope as new tools of measuring these changes become available. Fundamental questions (e.g., how best to quantify changes) become enigmatic with DNA methylation, histone modifications, and microRNA epigenetic modifications that can affect imprinted, coding, non-coding, and global regions of the genome. Understanding these questions is important in interpreting species/strain-specific responses. This advanced course is a practical guide to techniques used in epigenetic research with respect to toxicology for *in vivo/ex vivo* screening of rodent models, post-fertilization embryos, developmental biology, and human disease states. Topics range from advancements in techniques to screening strategies and tools, and include techniques to correlate epigenetic changes to gene expression and apical end points, use of imprinted genes as biomarkers, and profiling DNA methylation in human population-based research. For screening tools to determine species-specific responses, a variety of novel technologies will be analyzed such as epigenomic profiling of DNA methylation in mouse tumors, pyrosequencing to examine the activity of endogenous retroviruses (e.g., IAP), and assays to explore miRNA and histone modification changes. In addition, cutting edge techniques such as deep sequencing technologies of bisulfite-converted DNA will be discussed as these have enabled the characterization of methylation changes at the genome level; however, the significant challenge in using this technology is dealing with the massive amount of information obtained and making sense of the observed methylation changes. Scientists in academia, industry, and government will leave this course with an understanding of the strengths and weaknesses of available epigenetic tools, how these tools can be best used in screening and mode-of-action experiments, as well as insight into future potential of mechanistic epigenetic toxicology.

CE 13 QUANTITATIVE *IN VITRO* TO *IN VIVO* EXTRAPOLATION: THE ESSENTIAL ELEMENT OF *IN VITRO* ASSAY BASED RISK ASSESSMENT.

H. J. Clewell[1] and B. J. Blaauboer[2]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [2]*Utrecht University, Utrecht, Netherlands.*

There is increasing recognition of the need to use efficient approaches to assess the risk assessment of high numbers of chemicals in a short time. The reliance on approaches consisting of animal experimentation has its drawbacks in terms of ethical, economical, and – not least – scientific limitations in assessing risks in a high-throughput mode. The quantitative interpretation of toxic effects of compounds in *in vitro* studies, using *in silico* approaches such as systems biological descriptions of toxicity pathways and physiologically based pharmacokinetic modeling (PBPK), are a necessary component of the National Academy of Sciences vision on toxicity testing in the 21st Century. The limited studies performed with this approach to date have shown that good predictions for the risk of the use of chemicals can be made. However, a number of limitations have also become clear and more standardization of methods is needed before implementation of quantitative *in vitro-in vivo* extrapolations (QIVIVE) in risk assessments can be achieved.
In this course, the following elements of the approach for assessing risks on the basis of *in vitro* toxicity data will be discussed:
• How can we improve the applicability of *in vitro* methods by determining the real concentrations that come into contact with the cells *in vitro*, both for chemical compounds and for particles?
• How can we effectively and efficiently integrate the metabolism of compounds, for clearance as well as for bioactivation?
• How can we provide a flexible and yet robust scheme for integrating the different elements in a high-throughput environment?

CE 14 STEM CELLS UTILITY IN TOXICOLOGY SCREENING.

M. M. Sebastian[1] and Z. A. Radi[2]. [1]*Columbia University, New York, NY and* [2]*Pfizer Global Research and Development, Cambridge, MA.*

The development of toxicological screening tools for evaluating toxicity of new drug candidates has been a major focus in the pharmaceutical industry. Human embryonic stem (hESC) cells and induced pluripotent stem (iPS) cells and their lineage cells can be used as tools to predict developmental and other toxicities of drug candidates since several of the human biochemical pathways are active in these cells. In addition, stem cells can also be used to help in the mechanistic understanding of how a specific class of compounds leads to toxicity. Participation in this course will provide a basic overview of the utility of stem cells in drug discovery and update toxicologists on a variety of stem cells applications as screening tool for evaluating toxicity in multiple organ systems, thereby giving toxicologists a better understanding of the potential practical application of these *in vitro* methods for safety and risk assessment.

CE 15 THE BIOLOGY AND TOXICOLOGY OF THE PERI- AND POST-NATAL DEVELOPMENT.

G. D. Cappon[1] and G. J. Chellman[2]. [1]*Pfizer Global Research and Development, Groton, CT and* [2]*Charles River Laboratories, Reno, NV.*

The susceptibility to toxicity of organ systems during *in utero* and post-natal development is a concern for both drugs and environmental chemicals. While developmental toxicity can be manifested by death, structural abnormalities, and altered growth, alterations in the functional competence are of special concern during post-natal development. The primary focus in the past has been on functional toxicity to the CNS and reproduction, but the potential for developmental exposure to impact function of other systems such as the cardiovascular, respiratory, immune, endocrine, and digestive systems is now widely recognized. This basic course will begin with a review of post-natal development of major organ systems in humans and how those developmental processes might translate to sensitive periods for toxicity. Focus will be placed on study designs for evaluation of pharmaceuticals during the pre- and post-natal development period and designs for juvenile animal toxicity studies to support pediatric drug development. Next, designs will be presented for assessment of post-natal and juvenile toxicity studies in non-human primates, a rapidly expanding area given the increase in biopharmaceutical research. The course will wrap up with a discussion of multigenerational studies used to assess potential toxicity of environmental chemicals. Attendees will leave with an appreciation of the complex biology of peri- and post-natal development periods and an overview of current approaches to evaluating safety during this period.

S 16 EMERGING ISSUES AT THE INTERSECTION OF REPRODUCTIVE AND MIXTURES TOXICOLOGY.

V. S. Wilson[1] and C. V. Rider[2]. [1]*Reproductive Toxicology Branch, TAD, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC and* [2]*Toxicology Branch, NIEHS-NTP, Research Triangle Park, NC.*

Environmental contaminants have been implicated as having a role in reproductive toxicity observed in both humans and wildlife. For example, estrogenic and antiandrogenic chemicals have been hypothesized to be involved in the observed rise in the incidence of testicular dysgenesis syndrome noted as a suite of related pathologies including decreased semen quality, increased incidence of male reproductive tract malformations, and testicular cancer. Additionally, endocrine disruptors from concentrated animal feedlot operations and sewage effluent have been associated with observed reproductive anomalies in aquatic species. While toxicological studies and regulatory action have traditionally focused on individual chemicals, it is clear that realistic exposures are made up of multiple chemicals. Sources of exposure to endocrine active compounds are varied and include personal care products, pharmaceuticals, agricultural, and industrial compounds. Many of these sources may lead to constant, low dose exposures. Although individual chemicals are typically present at low levels within the exposure milieu, the impact of mixtures of these chemicals has only recently begun to be examined. Our panel of experts will provide an overview of potential exposures to endocrine active mixtures and describe current work on mixtures of reproductive and developmental toxicants from both a human health and ecotoxicological perspective. In conclusion, we will address emerging contaminants of concern and discuss the reproductive effects of relevant chemical mixtures in humans and wildlife.

S 17 BIOASSAY USE IN IDENTIFYING ENDOCRINE DISRUPTORS IN THE ENVIRONMENT.

D. Schlenk. *Environmental Toxicology Program, University of California Riverside, Riverside, CA.*

Advances in analytical chemistry have led to the detection of multiple chemical agents from numerous sources in surface waters around the globe. As such, the interactions of these mixtures continue to be an area of great uncertainty with regard to the potential risk of these "emerging" chemical agents to aquatic organisms, particularly extremely sensitive endocrine-specific responses. Using receptor-based models for estrogenic activity, strong correlations are observed with the occurrence of known estrogen receptor ligands and biological activity facilitating implementation of an EEQ approach based upon receptor additivity. However, when in vitro responses are partnered with in vivo activities, poor correlations are observed particularly in agricultural locations. Studies in the Central Valley of California using a combined approach of analytical chemistry with in vitro and in vivo bioassays for estrogenic activity in a Toxicity Identification Evaluation failed to identify concentrations of estrogen receptor ligands equivalent to either in vitro or in vivo EEQ values. Of more than a hundred analytes, only a few pesticides, alklyphenols (APs) and alkylphenol ethoxylates were detected in samples showing estrogenic activity. When detected compounds were administered in ambient concentrations as individual compounds, neither in vitro nor in vivo estrogenic activity was observed. However, when pesticides were combined with surfactants, similar EEQ values to those observed in field samples were observed in vivo, but not in vitro. Overall, these results indicate additivity-based models using in vitro methods may underestimate endocrine responses of mixtures in vivo, particularly when compounds have multiple targets along the hypothalamus-gonadal-pituitary axis (eg. APs).

S 18 DIAGNOSTIC ASSESSMENT OF THE ECOLOGICAL RISK OF EDCS IN COMPLEX MIXTURES.

G. Ankley. *Med., NHEERL, ORD, U.S. EPA, Duluth, MN.* Sponsor: L. Gray.

Although it is important to be able to forecast the potential endocrine toxicity of chemical mixtures that could enter aquatic environments, in many instances there is a need to determine possible effects of endocrine-active chemicals already present in complex environmental mixtures (e.g., effluents, sediments), as well as discern specific chemicals causing observed responses in exposed animals such as invertebrates, fish and amphibians. This presentation will focus on techniques to: (1) detect the occurrence of endocrine-active chemicals (e.g., estrogen and androgen receptor agonists) in complex aqueous samples using mechanism-based responses in cell lines or whole animals, (2) identify specific chemicals responsible for endocrine toxicity in complex mixtures through biologically-based fractionation procedures, and (3) use pathway-specific models to assess the potential for adverse effects of chemical mixtures on individuals and populations. Case examples illustrating these approaches will focus on the effects of complex municipal, industrial or agricultural discharges, and chemical components of these wastes

S 19 MOLECULAR ENDPOINTS AND MIXTURES OF ENDOCRINE DISRUPTING CHEMICALS IN FISH.

N. D. Denslow[1], N. Garcia-Reyero[2], K. J. Kroll[1], E. F. Orlando[3], K. H. Watanabe[4], M. S. Sepúlveda[5], D. L. Villeneuve[6], E. J. Perkins[7] and G. T. Ankley[6]. [1]*Physiological Sciences, University of Florida, Gainesville, FL,* [2]*Chemistry, Jackson State University, Jackson, MS,* [3]*Animal and Avian Sciences, University of Maryland, College Park, MD,* [4]*Division of Environmental and Biomolecular System, Oregon Health & Science University, Beaverton, OR,* [5]*Forestry and Natural Resources, Purdue University, West Lafayette, IN,* [6]*ORD, NHEERL, Med., U.S. EPA, Duluth, MN and* [7]*Environmental Laboratory, U.S. Army Engineer Research and Development Center, Vicksburg, MS.*

Microarray technology is a relatively novel tool in ecotoxicology and is beginning to be used for exposure and/or hazard characterization for ecological risk assessment. To develop a basis for this type of analysis, fathead minnows (Pimephales promelas) were treated with two binary mixtures: 17α-ethinylestradiol and the estrogen antagonist, ZM 189,154; and 17β-trenbolone (androgen) and the androgen antagonist, flutamide. Concentrations chosen were anchored to levels which affect cumulative egg production in 21-day fathead minnow reproduction assays. Unique gene expression fingerprints were identified in endocrine active tissues including brain, gonad and liver, which point to adverse outcome pathways. In the presence of excess antagonist, gene expression was reversed, but only for some genes, suggesting that the model compounds have additional activities beyond binding to soluble sex hormone receptors. Using the validated microarrays, effluents from water treatment plants and animal agricultural areas were tested. Expression profiles were unique at each of the locations, suggesting that they vary in their composition and complexity. Expression changes in key genes such as steroidogenic acute regulatory protein (StAR) and estrogen receptors, among others illustrate the presence of endocrine disrupting chemicals. Pathway analysis illustrates toxicity pathways that may be of importance for complex effluents.

S 20 EFFECTS OF MIXTURES OF PHTHALATES, PESTICIDES, AND TCDD ON SEXUAL DIFFERENTIATON IN RATS: A RISK FRAMEWORK BASED UPON DISRUPTION OF COMMON DEVELOPING SYSTEMS.

L. E. Gray. *Reproductive Toxicology Branch, U.S. EPA, Research Triangle Park, NC.*

Since humans are exposed to more than one chemical at a time, concern has arisen about the effects of mixtures of chemicals on human reproduction and development. We are conducting studies to determine the 1) classes of chemicals that disrupt sexual differentiation via different mechanisms of toxicity, 2) effects associated with in utero phthalate, pesticide and 2,3,7,8 dioxin (TCDD) exposures, and 3) how mixtures of phthalates behave when combined with pesticides or TCDD. In the mixture studies, we have examined the postnatal development of male rat offspring after in utero exposure to 1) pairs of AR antagonists, 2) pairs of phthalates, 3) phthalates with AR antagonists, 4) five phthalates, 5) seven chemicals (four pesticides and three phthalates), 6) ten chemicals (four pesticides and six phthalates) and 7) the potent Ah receptor agonist TCDD plus a phthalate. We also have examined the effects of some of these chemicals on fetal male rat hormone levels and testicular gene expression levels. Results of these studies demonstrate that dose addition models generally provide the most accurate predictions of the observed effects of these mixtures on male rat sexual differentiation. For example, when ten chemicals were administered in utero, 100% of the males displayed reproductive tract malformations as predicted by dose addition models, whereas response addition models predicted that none of the males would be malformed. Our data indicate that cumulative risk assessments based upon disruption of common fetal targets or systems during development more accurately predicts mixture effects than does one based upon mechanisms of toxicity. This abstract does not necessarily reflect EPA policy. NTP, NIEHS/EPA Interagency Cooperative Research Agreement HHS Y1-ES-8014-01; EPA RW75922

S 21 ESTROGENIC CHEMICAL MIXTURES AND ENDOGENOUS HORMONES – A ROLE IN BREAST CANCER DEVELOPMENT?

A. Kortenkamp and E. Silva. *Centre for Toxicology, The School of Pharmacy, University of London, London, United Kingdom.* Sponsor: C. Rider.

Elevated levels of endogenous steroidal estrogens have been linked to breast cancer risk, and there is good evidence that pharmaceutically administered steroids (for example as hormone replacement therapy) also play a role in the disease. The possible contribution of synthetic environmental estrogens remains controversial, in view of their low potency compared with estradiol. To assess whether synthetic estrogens could conceivably add to the internal estrogenic load of women, we have conducted in vitro experiments with mixtures of steroidal, plant-derived and synthetic estrogenic chemicals. The agents were combined at the levels reported to occur in women's serum, and assessed in the E-Screen assay. We found that the combined effects could be predicted accurately by using the concept of concentration addition. With extreme exposure scenarios similar to those encountered in certain areas of South Europe, there was a significant added contribution of synthetic estrogens, over and above that of endogenous estrogens. Persistent organochlorine compounds such as p,p-DDE, frequently measured in breast cancer epidemiology studies, contributed little to an overall combination effect. The contribution of more polar phenolic chemicals, such as those used as UV-filter substances, was more pronounced. Our studies may help to prioritise chemicals for study in breast cancer epidemiology.

S 22 ENVIRONMENTAL OXIDATIVE POLLUTANTS-INDUCED PULMONARY TOXICITY.

L. L. Mantell[1] and J. T. Zelikoff[2]. [1]*St. John's University College of Pharmacy, Queens, NY and* [2]*New York University School of Medicine, Tuxedo, NY.*

Environmental oxidant toxicants remain a major public health concern in industrialized cities throughout the world. Population and epidemiological studies have associated oxidant air pollutant exposures with morbidity and mortality outcomes, and underscore the important detrimental effects of these pollutants on the lung. A

well-orchestrated lung inflammation induced by cytokines is critical to optimizing host defense capabilities, while avoiding or minimizing potential damage to lung tissues. Normally, pulmonary inflammation plays a pivotal role during positive immune responses against microbial and small particle pathogens. Unfortunately, certain inhaled toxicants, such as asbestos, can non-specifically induce dysregulated chronic and acute inflammation within the respiratory tract. Our pannel of experts will emphasize and examine the types of immunomodulatory events that occur in the lungs in response to environmental agents and pathogens, and to demonstrate how these disparate challenges can lead to similar outcomes. In addition, cutting edge studies on the regulation of reactive oxygen species (ROS) production will be discussed. In conclusion, summaries will be provided that will allow for the recognition of the major putative mediators involved in induction of pulmonary inflammation; describe signaling pathways that mediate inflammatory responses to inhaled toxicants and pathogens; characterize reactive oxygen/nitrogen species (ROS/RNS) from exposure to environmental toxicants; better understand the interrelationship between ROS/RNS, inflammatory responses and their impact on diseases; and, identify potential targets and therapeutic strategies for further development for the amelioration of acute lung inflammatory injury and chronic diseases.

S 23 A SHORT OVERVIEW ON ENVIRONMENTAL OXIDATIVE POLLUTANTS-INDUCED PULMONARY TOXICITY.

L. Mantell. *Pharmaceutical Sciences, St. John's University/The Feinstein Institute for Medical Research, Queens, NY.*

Environmental oxidant toxicants remain a major public health concern in industrialized cities throughout the world. Population and epidemiological studies have associated oxidant air pollutant exposures with morbidity and mortality outcomes, and underscore the important detrimental effects of these pollutants on the lung. A well-orchestrated lung inflammation induced by cytokines is critical to optimizing host defense capabilities, while avoiding or minimizing potential damage to lung tissues. Normally, pulmonary inflammation plays a pivotal role during positive immune responses against microbial and small particle pathogens. Unfortunately, certain inhaled toxicants, such as asbestos can non-specifically induce dysregulated chronic and acute inflammation within the respiratory tract.

S 24 PRO-INFLAMMATORY RESPONSES TO OXIDIZED MATRIX COMPONENTS IN RESPONSE TO ASBESTOS INJURY.

T. Oury. *Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA.*
Sponsor: L. Mantell.

Asbestos exposure is known to cause several pulmonary diseases including asbestosis, lung cancer, and mesothelioma. Inhalation of asbestos fibers is believed to induce tissue injury by altering the oxidant/antioxidant balance in the lung and pleural tissue. This oxidant/antioxidant imbalance results from the direct induction of oxidant production due to redox available iron on the surface of the asbestos fibers as well as by inducing oxidant generation by inflammatory cells that respond to the inhaled fibers. This increased production of oxidants is thought to directly contribute to these diseases as several studies have shown that antioxidant enzymes such as catalase and superoxide dismutases are able to protect against asbestos-induced lung injury. Much of the increased oxidant production induced by asbestos exposure occurs extracellularly. Thus, extracellular targets of oxidative injury are likely to contribute to asbestos-induced lung injury. Dr. Oury will discuss several targets of oxidant-induced injury in the extracellular matrix that may contribute to asbestos-induced inflammation and lung injury and how the enzyme extracellular superoxide dismutase functions to inhibit this process.

S 25 USE OF OXIDATIVE LIPIDOMICS AND SIGNALING BY OXIDIZED LIPIDS IN SAFETY SCREENING OF NANOPARTICLES.

V. E. Kagan. *Environmental and Occupational, University of Pittsburgh, Pittsburgh, PA.*

Engineered nanomaterials are becoming a pervasive presence in different spheres of modern life – from diverse technological applications to a plethora of consumer products – and this raises concerns about their possible adverse effects on human health and the environment. Among the different nanomaterials available today, carbon nanotubes (CNT), particularly single-walled carbon nanotubes (SWCNT), are most advanced and abundant in their applications. The unique physico-chemical characteristics combined with the vast surface area make the biological effects of CNT and their interactions with cells largely unpredictable. Therefore, the cytotoxicity of these novel nanomaterials cannot be readily deduced from previous investigations of other particles or fibers. In fact, in vitro data indicate that SWCNT may exert strong cytotoxicity with induction of oxidative stress. More importantly, recent in vivo studies have demonstrated robust and unusual pulmonary inflammatory responses of SWCNT upon exposure of mice via aspiration or inhalation. Inflammatory tissue responses were also observed when certain multi-walled CNT with high aspect ratios were administered to mice via intraperitoneal injection. Notably, myeloperoxidase of inflammatory cells, particulalry of neutrophils, is capable of effective CNT biodegradation to CO2 whereby both hypochlorous acid and reactive intermedates of the enzyme contribe to oxidative modification of CNT. Using an established mouse model of pharyngeal SWCNT aspiration, Dr. Kagan and colleagues showed that biodegradation of carbon nanotubes nullified their ability to induce characteristic pulmonary inflammatory responses. These findings strongly indicate that numerous novel biomedical applications of carbon nanotubes may be achievable under conditions of carefully controlled biodegradation.

S 26 DIFFERENTIAL MECHANISMS OF SUSCEPTIBILITY TO OXIDANT-INDUCED LUNG DISEASE.

E. M. Postlethwait. *Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.*

Environmental oxidant exposures remain a major public health concern. Epidemiologic studies have associated oxidant air pollutants with diverse health impacts, including pulmonary and cardiovascular, which underscores the continued need to further characterize mechanisms that govern induction of and susceptibility to exposure-related pathobiologies. Children experience enhanced dosimetry during outdoor activities, which is superimposed on growth and development. Thus myriad biological processes may be affected with a potential for long-term consequences. We have utilized a multi-disciplinary approach to study site-specific ozone effects in infant non-human primates, as their lung development more closely resembles humans than do rodents. Results to date have shown abnormalities with regard to adaptive responses in nose and lung, cardiovascular perturbations, minimal surface antioxidant compensatory responses, atypical inflammation, and disruptions in normal airway development. Persistent atypical response to both ozone and LPS exposure are observed even after 6-9 months filtered air recovery including inflammatory and TRL agonist challenge in circulating cells. Global mRNA expression analyses of site specific (axial, terminal bronchioles) gene expression using Ingenuity Pathway Analyses (IPA) identified differential patterns involving immune, inflammatory and developmental systems. Ongoing investigation of global DNA methylation in the same tissues may provide further gene expression insights. Hence, an investigational model that approximates translational studies as closely as possible has shown that early life exposures result in unique and persistent sequelae, suggesting that childhood exposures may appreciably influence disease pathogenesis and susceptibility to a spectrum of environmental challenges during later life.

S 27 TARGETING INFLAMMATION AND THE INFLAMMASOME USING ANAKINRA, A POTENTIAL THERAPEUTIC AGENT FOR MESOTHELIOMA.

B. Mossman. *Pathology, University of Vermont College of Medicine, Burlington, VT.*
Sponsor: L. Mantell.

Chronic inflammation has been linked to the development of several cancers, including malignant mesothelioma (MM). Dr. Mossman's laboratory has shown that inflammation by asbestos fibers is governed by a multi-protein complex termed the inflammasome which is linked to release of several pro-inflammatory proteins called cytokines (IL-1β, IL-18, and IL-33) from human macrophages, i.e. cells of the immune system that may augment the development of inflammation and MM (1). Exciting recent experiments indicate that activation of the inflammasome by asbestos fibers occurs in human mesothelial cells in culture and during the development of human peritoneal MMs in an immunocompromised mouse xenograft model. Moreover, increased amounts of a number of pro-inflammatory (including IL-1β) and tumor-promoting cytokines can be detected with changes in inflammatory cell types in peritoneal lavage fluids of these mice. We have used these models and the IL-1β receptor blocker, anakinra, to block cytokine release from human MM cells, and studies with this drug to inhibit inflammation and MM development are in progress. These experiments show that anakinra is nontoxic after repeated administration to mice and abrogates cytokine production associated with tumor development. Currently, anakinra is being tested alone and in combination with a multi-kinase inhibitor, ZD6474, in a novel multi-targeting strategy, using localized intracavitary administration, to inhibit the growth of human MMs in mice. These combined therapy approaches will be discussed as attempts to prevent both chronic inflammation by asbestos and increases in survival pathways (MAPK, AKT) that have been associated with asbestos exposures and chemoresistance.

S 28 HIGH CONTENT IMAGING – APPLICATIONS IN TOXICOLOGY AND TOXICITY TESTING.

W. Mundy. *Integrated Systems Toxicology Division, U.S. EPA, Research Triangle Park, NC.*

High Content Imaging (HCI) often referred as high content screening (HCS), combines state-of-the-art microscopy, robotics, and computer-assisted image analysis to measure multiple cellular and sub-cellular features. The technology produces information (digital images and metadata) on cellular response or morphological changes at a high level of detail. Assays are amenable to high-throughput automation generating hundreds of measurements per cell and millions of data points from a single microtiter plate. Introduction of this technology in the pharmaceutical industry led to analysis of cell signaling and processes such as receptor internalization. The technology has expanded to include applications in angiogenesis, wound healing, cell cycle regulation, cell death, neurodegeneration, regeneration, and genotoxicity, etc. Using these approaches, measurements are made at the single cell or population level. HCI assays are generally conducted using cell lines, primary cells, and stem cells. *Ex vivo* tissue and whole organisms such as *Drosophila, C. elegans*, and zebrafish can also be imaged providing knowledge from intact specimens. Novel strategies for toxicity testing are likely to expand with HCI studies examining biologically significant cellular perturbations by linking genetic, phenotypic, or functional cellular changes with adverse outcomes from exposure to environmental chemicals or pharmaceutical compounds. HCI is likely to become a key technology for developing toxicity pathway assays as outlined by the 2007 NRC report Toxicity Testing in the 21st Century: A Vision and A Strategy. Furthermore, it is now evident toxicology programs in the pharmaceutical industry have adopted this technology in an effort to expedite the fate of compound candidate selection in preclinic and clinical trials using key measurements generated with HCI. Therefore it is important to provide an overview of this technology and its applications in toxicity testing relative to the HCI field from academia, biopharma, and government agencies with examples and case studies to gain knowledge and insight into toxicity pathways.

S 29 AN INTRODUCTION TO HIGH CONTENT IMAGING TECHNOLOGIES AND APPLICATIONS IN TOXICOLOGY.

O. Trask. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.* Sponsor: R. Thomas.

High content imaging (HCI) technology has provided great promise to the scientific community with a plethora of the different instrumentation platforms covering a vast array of biological applications to study disease including signaling cascades, MOA, and phenotypic characterization in immortalized and primary cells, ex-vivo tissue, and small organisms such as zebrafish, C. elegans and drosophila. From the onset HCI has bestowed investigators with a number of underlying cellular measurements which have been exploited to determine cell death/loss and toxicity assessments in multiple disciplines across the biopharmaceutical industry, academia and government agencies. An introduction and broad stroke overview of commonly used HCI assays in toxicology such as micronucleus, comet assay, apoptosis, cell loss, functional biomarkers, and others will be covered. An often overlooked aspect of HCI technology occurs during data analysis of primary endpoint assays, the other "high content" or multiparametric data is not commonly used. I will provide the audience with key examples how embedded data is extracted from digital images at a single cell level or as global population statistics to measure features such as DNA content or cell loss as an indicator of cytotoxicity during compound treatment respectively. To a degree with the exception of flow cytometry these key secondary high content measurements are not typically obtained by other cell based technologies. It is well known with all technologies there are limitations, knowing the caveats of the technology and approaches are critical for a productive outcome including the importance of institutional infrastructure to support the implementation of the technology is a key component for success.

S 30 HIGH CONTENT ANALYSIS OF CYTOTOXICITY FOR PREDICTION, MECHANISTIC ELUCIDATION, AND BLOOD BIOMARKERS OF HUMAN TOXICITY.

P. J. O'Brien. *University College Dublin, Belfield, Dublin, Ireland.* Sponsor: J. Trask.

High content analysis (HCA) of in vitro biochemical and morphological effects of drugs and chemicals is concordant with potential for human toxicity. Concordance was greater for cytotoxic effects assessed by HCA than for conventional cytotoxicity tests and for regulatory animal toxicity studies. Additionally, HCA identified chronic toxicity potential, and drugs producing idiosyncratic adverse reactions and / or toxic metabolites were also identified by HCA. Mechanistic information on the subcellular basis for the toxicity is frequently identified, including various mitochondrial effects, oxidative stress, calcium dyshomeostasis, phospholipidosis, apoptosis, and antiproliferative effects, and a fingerprinting of the sequence and pattern of subcellular events. As these effects are frequently non-specific and affect many cell types, some toxicities may be detected and monitored by HCA of peripheral blood cells, such as for anticancer and anti-infective drugs. Critical methodological and interpretive features are identified that are critical to the effectiveness of the HCA cytotoxicity assessment, including, the need for multiple days of exposure of cells to drug, use of a human hepatocyte cell line with metabolic competence, assessment of multiple prelethal effects in individual live cells, consideration of hormesis, the need for interpretation of relevance of cytotoxicity concentration compared to efficacy concentration, and quality management. Limitations of the HCA include assessment of drugs that act on receptors, transporters, or processes not found in hepatocytes. HCA may be used in a) screening lead candidates for potential human toxicity in drug discovery alongside of in vitro assessment of efficacy and pharmacokinetics, b) monitoring in vivo toxicity of drugs with known toxicity of known mechanism.

S 31 QUANTITATIVE *IN VITRO* MEASUREMENT OF CELLULAR PROCESSES CRITICAL TO THE DEVELOPMENT OF NEURAL CONNECTIVITY USING HCA.

J. A. Harrill. *Integrated Systems Toxicology Division, U.S. EPA, Research Triangle Park, NC.*

New methods are needed to screen thousands of environmental chemicals for toxicity, including developmental neurotoxicity. In vitro, cell-based assays that model key cellular events have been proposed for high throughput screening of chemicals for developmental neurotoxicity. While in vitro systems can not fully replicate the complex temporospatial development of the brain, neuronal cultures can recapitulate neurodevelopmental processes such as cell proliferation, differentiation, growth, and synaptogenesis.We have evaluated primary neural cultures as models for neurite outgrowth, estabalishment of polarity and synaptogenesis: processes critical for the development of neuronal connectivity. In primary neural cultures, these events occur sequentially, and the effects of chemicals on specific neurodevelopmental processes can be investigated using different developmental / exposure periods. This talk will focus on the development of high content assays which allowed concentration-response assessments of multiple chemicals in 96 well microtiter plates, in an efficient and cost-effective manner. The ability of these assays to detect effects on in vitro neural development was determined using a "training set" of chemicals with known effects in vitro. Specific effects on neurodevelopment processes could be separated from cytotoxic effects using parallel analysis of cell number and morphological measurements. The results demonstrate that HCA assays can rapidly quantify chemical effects in vitro, distinguish between specific effects on neurodevelopmental endpoints and nonspecific changes in cell health and provide comparative data on which developmental events are more sensitive to a particular chemical insult. This abstract does not necessarily reflect USEPA policy.

S 32 BEYOND DECREASING ATTRITION: HIGH CONTENT IMAGING (HCI) IN GENETIC TOXICOLOGY.

E. Rubitski. *Genetic Toxicology, Pfizer, Groton, CT.* Sponsor: J. Aubrecht.

HCI is transforming the way in which in vitro clastogenicity screening & mechanistic follow up studies are performed in Genetic Toxicology. The In Vitro Micronucleus (IVMN) assay has been used for years as a screening tool to predict the aneugenic and clastogenic potential of chemical agents. Regulatory agencies have recently approved the IVMN assay (OECD Guideline 487) as an alternative for both the GLP (Good Laboratory Practices) Human Lymphocyte Assay and Mouse Lymphoma assays. Since there are advantages to running the assay both with and without cytochalasin-B (CYB), e.g. to increase sensitivity and specificity for certain classes of compounds, and because we have two state of the art automated IVMN platforms capable of evaluating micronuclei in both mononcleated and binucleated Chinese Hamster Ovary cells, we have evaluated extensive dose-response relationships for 11 commercially available chemicals. The chemicals included in our study were suggested in the guideline for validation. Here we report and contrast the results of each chemical within the context of each IVMN platform (+/- CYB) and the OECD Proposed Guideline 487. The results of this study show that the automated system allows for rapid and consistent quantification of DNA damage with results that are concordant with previous manual studies de-

scribed in the literature. We have optimized the way in which users can view and evaluate the automated results. Commercially available software is cumbersome for quickly evaluating both the overall health of a particular culture and cell level details of the entire population. This step is crucial for evaluating pharmaceutical compounds in development as each class of compound causes various morphologies which can tax the ability of the automated algorithm. Our interface generates well level images and individual thumbprints of cells in question. We can review the calls made by the system and make changes which are then immediately reflected in the output data. These changes can be tracked for audit. Now that the operational performance for DNA damage detection is optimized, HCI is providing us with a multitude of ways to follow up positive results (i.e. cytoskeletal rearrangement, centrosome enumeration). It also affords us the ability to evaluate the individual mechanism(s) by which test chemicals cause damage. This thorough understanding provides for high confidence in our risk management strategies.

S 33 HIGH CONTENT SCREENING (HCS) IN EARLY SAFETY ASSESSMENT: FROM DATA TO PREDICTIVE MODELS.

M. Kansy[1] and A. Hoffman[2]. *[1]Non-Clinical Safety, F. Hoffmann-La Roche Ltd., Basel, Switzerland and [2]Discovery Technologies, F. Hoffmann-La Roche Ltd., Nutely, NJ.*

High content screening (HCS) enables the simultaneous determination of multiple features of molecular phenotypes which are assumed to be relevant to therapeutic and toxic activities of compounds. High content screening has been used to a larger extend in ligand-target prediction to identify or confirm mechanism of actions. In addition HCS approaches have been used in identifying potential toxic liabilities of drugs and drug candidates. Validation studies evaluating HCS as predictive tool in toxicity prediction were usually performed with relatively small data sets of toxic and assumed "non-toxic" compounds. Published validation studies on larger compound sets including hundreds of compounds are rarely described in literature. However, the application of HCS in the lead optimization phase for toxicity assessments and compound selection/optimization is assumed to positively impact the identification of high quality candidate molecules in early drug discovery. We will describe the result of our efforts to evaluate High Content Imaging (HCI) approaches in their capability to classify toxic effects of development and marketed compounds. Based on safety profiles determined in rats, more than 235 compounds were grouped into toxicity severity classes (high, medium, low). The effect of different compounds at concentrations from 0.3 to 100 µmolar on HEPG2 cells was determined in vitro by applying HCI. More than 60000 data points were generated. In order to separate toxic from non toxic compounds, obtained data were analyzed with computational tools and methods like e.g. Naive Bayesian, support vector machine (SVM) classification, kernel ridge regression (Kridge) and specific multivariate data analysis tools. The analysis indicated a weak but significant relationship between HCI observations and rat overall toxicity, allowing a separation of toxic from non toxic compounds. Derived models can be obtained with a much smaller set of HCS observations, usually cell viability parameter, without significant loss of accuracy.

S 34 RIBOTOXIC STRESS: MECHANISMS AND MODELS FOR HUMAN DISEASE.

J. J. Pestka[1], V. L. Tesh[2], B. E. Magun[3], Y. Moon[4] and N. Tumer[5]. *[1]Michigan State University, East Lansing, MI, [2]Texas A&M University Health Science Center, College Station, TX, [3]Oregon Health and Science University, Portland, OR, [4]Pusan National University School of Medicine, Yangsan, Republic of Korea and [5]Rutgers University, New Brunswick, NJ.*

Many xenobiotics evoke toxicity and cause disease by modifying critical mitogen-activated protein kinase (MAPK) signaling pathways that regulate growth, differentiation, and cell survival. A number of plant, fungal, bacterial, and algal toxins can aberrantly activate the P38, ERK, and JNK MAPKs by targeting the ribosome via a process termed the ribotoxic stress response. Examples of ribotoxic agents include natural toxins produced by plants (ricin), fungi (trichothecenes), bacteria (Shiga toxins), and algae (palytoxin). These agents can be encountered in food and water and are of further concern because of their potential use in chemical terrorism. Cells involved in the innate immunity appear to be particularly sensitive to ribotoxic stress. Ribotoxic stress is not completely understood but possible mechanisms activation of intracellular signaling pathways by sensors of damage-associated molecular patterns (DAMPs) or endoplasmic reticulum stress. From a translational perspective, exposure of experimental animals to ribotoxic stressors can result in downstream pathologic sequelae that remarkably mimic clinical signs associated with inflammatory human diseases such as acute respiratory distress, ulcerative colitis, IgA nephropathy, and hemolytic uremic syndrome. To address these issues, our panel of experts will explore commonalities and differences in upstream mechanisms of ribotoxic stress by different biotoxins and relate these to downstream sequelae associated with human inflammatory diseases.

S 35 RICIN MEDIATES ACUTE RESPIRATORY DISTRESS THROUGH IL-1BETA.

B. Magun. *Oregon Health and Science University, Portland, OR.* Sponsor: J. Pestka.

Pulmonary toxicity induced by aerosolized ricin is characterized by accumulation of inflammatory cells and destruction of alveoli. Ricin-induced morbidity is strongly reduced in mice that lack IL-1 or IL-1R, or in mice receiving recombinant IL-1 receptor antagonist (IL-1RA), demonstrating a key role of IL-1 in orchestrating inflammatory responses. Ricin-mediated activation of JNK and p38 through ZAK, a MAP3K, is responsible in part for the proinflammatory effects of ricin. We have identified two inhibitors of ZAK that are currently employed by as cancer therapeutics and that have very limited toxicity in humans. In addition, ricin activates the NALP3 inflammasome in macrophages through a signaling pathway that does not involve the activation of ZAK, JNK, or p38. Our evidence suggests that all inhibitors of protein translation will activate the NALP3 inflammasome in macrophages, suggesting a novel role of translation inhibition in mediating inflammatory responses.

S 36 MUCOSAL RIBOTOXIC STRESS AND INTESTINAL INFLAMMATORY DISEASES.

Y. Moon. *Department of Microbiology and Immunology, Pusan National University School of Medicine, Yangsan, Republic of Korea.*

Inflammatory bowel diseases (IBD) are aggravated by disrupted epithelial barrier integrity and the following microbial translocation. Many muco-active ribotoxic stress agents are known to cause IBD-like symptom by triggering pro-inflammatory responses and mucosal intolerance to the dietary xenobiotic. Studies are aimed at addressing the potential mechanistic association of the chemical ribotoxic stress with human intestinal inflammatory diseases. There will be special focus on epithelial recognition of the mucosal ribotoxic stress in the complex gut environment and its subsequent contribution to the disease progress in human intestine (This work was supported by the Korea Research Foundation (KRF) grant funded by the Korea government (MEST) (No. 2009-0087028)).

S 37 SHIGA TOXINS AND THE HEMOLYTIC UREMIC SYNDROME.

V. L. Tesh. *Microbial and Molecular Pathogenesis, Texas A&M University Health Science Center, College Station, TX.* Sponsor: J. Pestka.

Shiga toxins are a family of genetically and structurally conserved proteins expressed by the enteric pathogens Shigella dysenteriae serotype 1 and certain serotypes of Escherichia coli. The capacity of Shiga toxin-producing bacteria to cause widespread disease is highlighted by recent outbreaks of bloody diarrhea associated with the ingestion of contaminated beef products or spinach. Unfortunately, 10-15% of patients with bloody diarrhea will develop life-threatening complications, chief among them acute renal failure (hemolytic uremic syndrome). Shiga toxins possess an AB5 structure with a single A-subunit in non-covalent association with a pentameric ring of B-subunits. Shiga toxins bind to human cells through B-subunit interaction with the membrane glycolipid globotriaosylceramide. Following toxin binding and internalization, the holotoxin is routed to the endoplasmic reticulum (ER) where an A-subunit fragment retrotranslocates into the cytoplasm and cleaves a single adenine residue from the 28S rRNA subunit, thereby mediating protein synthesis inhibition. More recently, Shiga toxins have been shown to be signaling molecules par excellence. In addition to inhibiting protein synthesis, Shiga toxins activate all major mitogen-activated protein kinase (MAPK) pathways and the Src/PI3K/Akt/mTOR pathway in human macrophages or macrophage-like cell lines. Activation of these pathways is involved in both the positive and negative regulation of cytokine expression in response to intoxication. The toxins also activate the ER stress response, a "quality control" system which normally monitors levels of mis-folded proteins in the ER lumen. Shiga toxin-mediated activation of this system triggers intracellular signaling events that result in apoptotic cell death. Signaling pathways activated in human macrophages by Shiga toxins will be reviewed along with potential roles of toxin-induced signaling in pathogenesis. Disruption of these signaling pathways may provide targets for future interventional strategies to prevent or ameliorate disease.

S 38 ROLE OF RIBOTOXIC STRESS AND INNATE IMMUNE ACTIVATION IN TRICHOTHECENE-INDUCED IGA NEPHROPATHY.

J. J. Pestka. *Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.*

Prolonged dietary exposure to the 8-ketotrichothecene mycotoxins deoxynivalenol (DON) and nivalenol (NIV) in the mouse induces aberrant polyclonal IgA upregulation and subsequent kidney IgA deposition in a manner that mimics the early stages of human IgA nephropathy (IgAN). A critical underlying mechanism for DON-induced IgA hyperproduction is systemic upregulation of IL-6 via transcriptional and post-transcriptional mechanisms. Induction of IL-6 and other proinflammatory genes is mediated by activation of mitogen-activated protein kinases in macrophages and monocytes - effects that can be recapitulated in intact animals. One initiating mechanism for the DON-induced ribotoxic stress response involves the activation of constitutive ribosome-associated protein kinases (1)double-stranded RNA (dsRNA)-activated protein kinase (PKR) and (2)hematopoetic cell kinase (Hck), a non-receptor associated Src oncogene family kinase. A second mechanism involves autophagy of the chaperone GRP78 with consequent activation of the ER stress response. A coherent model will be presented for the sequence of molecular events at the ribosome level that modulate intracellular signaling, drive IL-6 induction and ultimately induce aberrant IgA upregulation.

S 39 RICIN AND SHIGA TOXINS INTERACT DIFFERENTLY WITH THE RIBOSOMAL STALK.

N. Tumer[1], J. Chiou[1], X. Li[1], M. Remacha[2] and J. P. Ballesta[2]. [1]*Rutgers University, New Brunswick, NJ and* [2]*Universidad Autonoma de Madrid, Madrid, Spain.* Sponsor: J. Pestka.

The A subunits of Shiga-like toxins (Stx1 and Stx2) and ricin (RTA) are N-glycosidases that specifically remove an adenine from the α–sarcin/ricin loop (SRL) of the large rRNA, resulting in inhibition of protein synthesis. In our previous work, we showed that P proteins of the ribosomal stalk are critical for RTA to depurinate the SRL in yeast. The interaction between RTA and ribosomes did not follow a simple 1:1 interaction model and was characterized by a two-step binding model. Using purified, in vivo assembled, ribosomal stalk complexes from yeast, we showed a direct interaction between RTA and the isolated stalk complex that fit a simple 1:1 interaction model. Therefore, the interaction between the isolated stalk and RTA represented one of the two binding steps of the two-step binding model. We showed that P proteins of the ribosomal stalk are also critical for Stx1 and Stx2 to depurinate the SRL in yeast. Stx1 depurinated ribosomes less efficiently than RTA or Stx2 in a yeast strain with a modified stalk (ΔAB), in which P protein binding sites are deleted, suggesting that cytosolic P1/P2 proteins stimulate the depurination activity of Stx1 when they are able to bind to the stalk. The addition of purified P1α/P2β heterodimer stimulated in vitro depurination of ribosomes from the ΔP1 strain, which contains the binding sites for P1/P2 heterodimers, but not ribosomes from the ΔAB strain. The stimulation was greater for Stx1 than for RTA or Stx2, indicating that cytosolic P1/P2 proteins are more critical for Stx1 to access the ribosomal stalk. Addition of the P1α/P2β heterodimer did not stimulate depurination of ribosomes from the ΔAB strain, indicating that the free P1/P2 proteins do not directly stimulate the enzymatic activity of the toxins. Instead, they appear to bind to the toxins in the cytosol and recruit them to the ribosomal stalk. These results provide the first evidence that differences in the interaction of RTA, Stx1 and Stx2 with the ribosomal stalk contribute to their relative depurination activity and cytotoxicity.

W 40 DISEASE PREVENTION: THE NEXT 50 YEARS.

H. Zarbl. *Environmental and Occupational Health Sciences Instituite, Robert Wood Johnson Medical School, Piscataway, NJ.*

The science of toxicology has had a tremendous impact on preventing adverse effects of environmental toxicants, consumer products, and drugs on human health. The Society of Toxicology played a pivotal role in developing the practice of toxicology as a profession and a research discipline. As the Society embarks on its next fifty years, new opportunities and challenges will no doubt change the role of toxicology in the improving human health. High- throughput genomic technologies are increasingly providing mechanistic insights into how exposure to toxicants interacts with other exposures, genetic background, diet, lifestyle, co-morbid disease and numerous other factors to modulate the disease risk. The Society thus has an opportunity to once again play a leading role in improving public health by not only preventing toxicity, but by developing approaches to predicting and preventing disease in those already exposed and, by identifying individuals or populations at increased risk from exposures. Therefore, the future of toxicology should include an increasing presence in disease prevention through biomarker based exposure and risk assessment, and individualized intervention, chemoprevention, and predictive toxicology. These emerging approaches will be reviewed to provide a roadmap for increasing the role of mechanistically based, predictive toxicology and 'omics technologies in disease prevention.

W 41 ASSESSING THE IMPACT OF INTER-INDIVIDUAL GENETIC VARIABILITY ON TOXICITY THROUGH TOXICOGENOMIC DATA.

I. Rusyn. *Environmental Science and Engineering, University of North Carolina, Chapel Hill, NC.*

In the field of toxicogenomics, little attention is paid to the fact that transcript expression is a heritable trait and that genetic control of mRNA levels may present a challenge for using genomics in population studies. Gene expression Quantitative Trait Locus (eQTL) mapping is one of the new tools to evaluate the association between transcript expression and genotype in order to find genomic locations that are likely to regulate transcript expression. The availability of both gene expression and high density genotype data has enabled eQTL mapping in animal and human populations. These analyses have contributed significantly to our understanding of the impact that SNPs have on the physiological processes in multiple tissues, in both animals and humans. Furthermore, these studies show that genetic regulation of gene expression is a key contributor to population diversity through previously unknown mechanisms. eQTL mapping is a key new approach in toxicogenomic analyses for the evaluation of the potential role of genetic polymorphisms in the role that environmental exposures may play in the pathogenesis of common diseases.

W 42 COMBINING THE GENOME WITH ITS EXPOSOME FOR MECHANISTICALLY-BASED DISEASE PREVENTION.

C. P. Wild. *International Agency for Cancer Research, Lyon, France.* Sponsor: H. Zarbl.

It has long been appreciated that both genes and environment play a critical role in the etiopathogenesis of disease. While genetic background can now be accurately and rapidly determined using high-throughput technologies, exposure assessment still faces the limitations inherent in questionnaire data or extrapolation from ambient toxicant levels, for example. However the availability of –omics technologies and other advances in laboratory sciences coupled with new insights into underlying disease mechanisms (e.g. epigenetics) present exciting opportunities to refine environmental exposure assessment. On this basis the concept of the exposome was proposed, representing the sum of all exposures from conception onward, to complement the genome as a critical quantitative measure on which to investigate disease risk and prevention. The presentation will introduce the concept of the exposome, evaluate its potential applications, limitations and outline the roles of epidemiologists and laboratory scientists in elucidating the components of the human exposome.

W 43 TOP-DOWN EXPOSOMIC STRATEGIES TO CHARACTERIZE THE HUMAN EXPOSOME.

M. Smyth. *University of California, Berkeley, CA.*

Researchers interested in the causes of disease rely upon increasingly sophisticated gene-wide association studies (GWAS) to evaluate genetic differences across populations, while using questionnaires to characterize environmental exposures. Because inaccurate and imprecise estimation of 'environmental' exposures (including stress, diet, and both exogenous and endogenous chemical exposures) can severely bias inferences regarding environmental causes of diseases and gene-environment (G x E) interactions, this imbalance in data quality between genetic and environmental factors is problematic. What we need are environmental analogs of GWAS to characterize the "exposome" and open the door to discovery of the environmental causes of disease. Currently researchers use bottom-up exposomics where they assume a priori that they know what to look for and try to measure it. In top-down exposomics you take an agnostic discovery-based view and look for patterns, then attempt to find out what those patterns reflect in terms of exposure. Using a combination of omic high-throughput technologies, it should be possible to characterize the "human exposome", in which exposures from all environmental sources (diet, stress, chemicals etc) are assessed. This top-down discovery-based approach should work because the technologies are available now but currently require too much material and are too expensive (similar to DNA sequencing 20

years ago). We will illustrate how increasing throughput, lowering cost and reducing sample requirements should be feasible through bioengineering approaches, such as lab-on-a-chip devices. If successful, the pay-off in disease prevention from studies that interleaf the human genome with the human exposome would be huge.

W 44 HIGH-THROUGHPUT METABOLOMICS FOR IDENTIFICATION OF QUANTITATIVE EXPOSURE BIOMARKERS.

D. Jones. *Emory University, Atlanta, GA.*

This presentation will present results using a top-down mass spectrometry-based profiling method (LC-FTMS) of plasma and other body fluids to study the exposome in humans. This method relies upon the high mass accuracy and mass resolution of ion cyclotron resonance mass spectrometry to detect and quantify chemicals based upon ion intensities of high-resolution mass/charge (m/z) features. The approach is useful for human plasma because >90% of low-molecular weight chemicals detected have unique elemental compositions, which decreases demands for chromatographic separation and mass fragmentation. The method provides measurement of 2500 m/z features in 10 microliter extracts of plasma in 10 min. The method is sufficiently reproducible and cost-effective for routine use to provide personalized measures of the exposome. Application of the method demonstrates the capability to distinguish endogenously generated chemicals from dietary and/or environmental chemicals. Chemicals which change in association with age, exercise, lean body mass and other demographic and behavioral factors are identified. Furthermore, some chemicals which increase with age and/or catabolic states have accurate mass m/z values which are consistent with identity as plasticizers, pesticides, fungicides, antibiotics, halogenated hydrocarbons and other environmental agents. The studies support the feasibility to use LC-FTMS, along with other –omic technologies to systematically define the exposome. Applications to identify environmental exposures and evaluate persistent chemical burdens within individuals will be discussed.

W 45 ROLE OF THE HUMAN MICROBIOME IN HUMAN ENVIRONMENTAL EXPOSURE AND DISEASE PREVENTION.

E. Holmes. *Imperial College, London, United Kingdom.* Sponsor: H. Zarbl.

Increasing recognition is given to the role of the human microbiome in maintaining health and in contributing to the etiology of a wide range of pathologies. This presentation will focus on characterizing the covariation of the microbiome with metabolic changes occurring as a response to adverse genetic or environmental challenges. Metabolic profiles of biological samples, generated through high resolution spectroscopic methods, contain a wealth of biochemical information. Compartments such as urine, fecal water and colonic contents all contain metabolites deriving from either gut bacterial species or their mammalian co-metabolites and therefore carry latent information as to the function of key members of the microbiome. Integration of metabonomic and metagenomic data, typically DGGE or 454 sequence data, via multivariate mathematical modeling allows inferences to be drawn regarding the role of microbiota at the family or species level in mammalian systems. Illustrations will be adopted from both animal models, such as germ free rats, and from human studies in pathological conditions and nutritional intervention studies.

W 46 PUBLIC HEALTH OPPORTUNITIES AND CHALLENGES IN DISEASE PREVENTION.

J. D. Groopman. *Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.*

The overarching goal of the exposome project is to identify, characterize and quantify the exogenous and endogenous exposures and modifiable risk factors that predispose and predict health effects across an individual's life span. This large environmental science project will foster the integration of the diverse scientific disciplines and technologies with the objective to measure all environmental exposures from conception onwards; including exposures from diet, lifestyle and endogenous sources, as a quantity of critical interest to disease etiology. These metrics will contribute to the implementation of individualized prevention strategies.

W 47 NEW APPROACHES FOR INTEGRATING TOXICOLOGICAL AND EPIDEMIOLOGICAL DATA TO BETTER INFORM RISK ASSESSMENT.

D. R. Juberg. *Dow AgroSciences, Indianapolis, IN.*

There is increasing scientific and regulatory interest in determining how to better integrate animal toxicological data and epidemiological evidence into the risk assessment process. This need stems from the growing body of literature on environmental epidemiology and the frequent disparity observed in human and animal data relative to reported outcomes. This workshop will identify the expectations and limitations that are inherent in animal and epidemiological studies and how existing and emerging tools, technologies, and study designs may be better utilized for scientifically-defensible and informed risk assessments. We will review review the challenges that exist with both data types (i.e., animal and epidemiologic) and identify the types of tools and data that would allow improved integration of epidemiology and experimental toxicology studies through exploration of a common marker of dosimetry that would facilitate cross-comparison of data sets. A perspective on study design and dosing regimes used in regulatory studies will be presented as well as review of advancements that improve our ability to compare animal data with known or modeled human exposures. Epidemiology study design and the opportunities and limitations that exist for simultaneously characterizing exposures and risks in humans will be reviewed. Insight on some of the new tools and technologies that enable a more refined analysis of biological plausibility and mode of action, both critical when assessing animal and human data for use in risk assessment, will be provided. Finally, advancements in exposure science within the context of some U.S. EPA projects and research aimed at utilizing multiple sources of information (i.e., exposure data, internal biomarkers, and PBPK modeling) for improvements in our knowledge on chemical exposures to humans will be discussed.

W 48 USING EPIDEMIOLOGY IN HUMAN HEALTH RISK ASSESSMENT: RECENT ADVANCES.

A. Lowit. *Office of Pesticide Programs, U.S. EPA, Washington, DC.*

This presentation will review strengths and weaknesses of experimental toxicology and epidemiology in the context of using both kinds of data in risk assessment. In experimental toxicology studies, the study conditions can be controlled and the level chemical exposure is known. However, unlike epidemiology studies which focus on humans at actual environmental exposures, experimental studies require species extrapolation and most often use high doses. One tool for consideration when integrating epidemiology data with in vivo and in vitro experimental toxicology data is the proposed use of the Bradford-Hill Criteria (as modified in the Mode of Action Framework) as a transparent, logical approach. This presentation will discuss the importance of human biomarker data in interpreting epidemiology study as these data provide the range of human exposures potentially associated with health outcomes. To provide a linkage between the epidemiology and experimental toxicology findings, human biomarker data are most useful when evaluated in the context of well-conducted animal pharmacokinetic studies. The value of well-developed physiologically-based pharmacokinetic (PBPK) models will be highlighted along with recent work and advancements in using probabilistic exposure models in combination with PBPK models to interpret epidemiology data and to estimate population-level biomarkers. This presentation will use case studies on atrazine and chlorpyrifos to elucidate all of these topics.

W 49 NEW APPROACHES TO TOXICOLOGY STUDY DESIGN: LINKING TESTING DOSE RESPONSE AND DOSIMETRY TO HUMAN EXPOSURE AND HEALTH OUTCOMES.

J. Bus. *The Dow Chemical Company, Midland, MI.*

Most animal toxicity studies of pesticides and industrial chemicals describe experimental doses in units of externally administered compound, e.g., mg/kg body weight or ppm in diet, air or drinking water. In traditional risk assessment practice, external doses in toxicology studies are compared to equivalent estimates of external human exposures (Margin of Exposure; MoE). However, with recent rapid advancements in human biomonitoring programs, an opportunity exists to significantly improve MoE evaluations through comparisons of internal (tissue) dose across these two types of studies. Thus, concentrations of chemical and/or relevant metabolite(s) in human blood and/or urine can be related to internal doses producing NOEL and LOEL responses in animal toxicity studies. The advantages to this approach are that human biomonitoring data, which more precisely reflect aggregate absorbed environmental doses, can be directly matched to equivalent internal dose units in animal toxicity studies while also significantly reducing cross-species

pharmacokinetic uncertainties. Studies with the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4D) have demonstrated how internal dose measurements can be routinely and effectively quantified from animal dietary toxicity studies, a common mode of administration for many chemicals. In addition, units of internal dose common to human biomonitoring and toxicity studies can be further related to health protective regulatory reference standard doses, e.g., RfDs and RfCs, by development of "biomonitoring equivalent" (BE) values. Developed from pharmacokinetic modeling, BEs represent estimated blood/urine concentrations resulting from exposures to RfD/RfC exposure standards (Aylward et al., EHP 118: 177, 2010). These refinements to comparative internal dose quantitation offer important dose/exposure context to informing toxicologic plausibility and interpretation of observations reported in epidemiology studies.

W 50 THE ROLE OF EPIDEMIOLOGIC RESEARCH IN RISK ASSESSMENT: SOME CHALLENGES AND OPPORTUNITIES.

S. A. Harris[1, 2, 3]. [1]*Population Studies and Surveillance, Cancer Care Ontario, Toronto, ON, Canada,* [2]*Occupational Cancer Research Centre, Toronto, ON, Canada and* [3]*Dalla Lana School of Public Health, Divisions of Epidemiology and Occupational and Environmental Health, University of Toronto, Toronto, ON, Canada.* Sponsor: D. Juberg.

Epidemiologic data obtained from well designed and executed human studies can inform the risk assessment process. Because of the nature of environmental and occupational exposures, most studies are observational in design and thus subject to numerous limitations; the primary limitation is typically the validity and reliability of exposure assessment. An overview of epidemiologic study design, with a focus on those studies most useful for risk assessment, will be presented. Traditional methods used for exposure assessment and absorbed dose measurement for registration and regulatory purposes will be reviewed, and contrasted with those typically used in epidemiologic research. Opportunities in exposure assessment in large occupational or environmental epidemiologic studies (such as the Ontario Health Study) and promising methods for incorporation of dose information into epidemiologic research will be discussed. Continued collaborative efforts amongst epidemiologists, exposure scientists, toxicologists, and risk assessors will be necessary to develop and apply the tools necessary to allow for the better integration of epidemiologic study results into the risk assessment process.

W 51 NEW TOOLS AND APPROACHES TO LINK TOXICOLOGICAL MODELS TO HUMAN HEALTH EFFECTS.

R. S. Thomas. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Due to limitations on human testing, the use of surrogate models and endpoints are necessary when studying the effects of environmental chemicals on human health. However, translating the results obtained using the surrogate models and endpoints to human health effects poses many unique challenges. The development of new tools and approaches for linking toxicological models with human health effects will be highlighted using two examples. The first example focuses on the incorporation of human dosimetry and exposure into high-throughput in vitro toxicity screening. In this example, in vitro assays were used to estimate human metabolic clearance and plasma protein binding for ToxCast chemicals. Computational in vitro-to-in vivo extrapolation methods were then used to estimate the human oral equivalent doses that would be required to produce steady state in vivo concentrations equivalent to in vitro AC50 values from the high throughput screens. These oral equivalents were compared with human oral exposure estimates to assess whether significant in vitro bioactivity occurred within the range of expected human exposure. The second example focuses on the translation of biomarkers of effect from rodent models to humans. In this example, circulating mRNAs were evaluated as biomarkers of hepatotoxicity. In a rat model, increases in liver specific mRNAs were observed in the cell-free plasma of rats treated with two different hepatoxicants. Characterization of the circulating mRNAs showed an association with both necrotic debris and encapsulation within microvesicles. The increase in circulating liver mRNAs was confirmed in human subjects following drug-induced liver injury demonstrating translation of the biomarkers from animals models to humans.

W 52 THE ITERATIVE ROLE OF EXPOSURE SCIENCE IN RISK ASSESSMENT.

R. Tornero-Velez. *U.S. EPA, Research Triangle Park, NC.* Sponsor: D. Juberg.

The mapping of the human genome and the emergence of tools in computational, informational, and molecular sciences has led to new possibilities for improving chemical risk assessment. For the first time, these advances allow for a high throughput and broad scale examination of the molecular and cellular targets of chemicals. In 2007, EPA launched ToxCast™ in order to rapidly and cost-effectively prioritize the toxicity testing of a large number of chemicals. Because hazard and exposure mutually determine risk, the implementation of toxicity screening has led to awareness of the need to develop exposure screening approaches. Furthermore, there is the need to integrate the two approaches to focus toxicity testing on those chemicals and endpoints, at those life-stages and via those exposure routes, most likely to present a risk of concern (Dellarco et al., 2010). Thus, exposure assessment should not be relegated to the fourth step in risk assessment (following dose-response, as specified by the 1983 NRC "Red book") but rather should be considered iteratively with allocation of resources as guided by a risk screening approach. Herein, I describe approaches that make use of two types of exposure survey data, the National Health and Nutrition Examination Survey (NHANES), a cross-sectional survey of population biomarkers, and national surveys of pesticide residues in homes and daycare facilities to inform development of risk assessment models for a group of pesticides. *EPA reviewed this work but it does not necessarily reflect official Agency policy.*

PL 53 ACUTE PULMONARY RESPONSES TO MWCNT INHALATION.

M. G. Wolfarth[1], W. McKinney[1], B. T. Chen[1], V. Castranova[1, 2] and D. W. Porter[1, 2]. [1]*HELD/PPRB, NIOSH, Morgantown, WV and* [2]*Physiology and Pharmacology, West Virginia University, Morgantown, WV.*

The large scale manufacture of multi-walled carbon nanotubes (MWCNT) suggests occupational exposures may occur. In order to investigate the pulmonary toxicity of MWCNT, male C57BL6/J mice (6 weeks old) were exposed to aerosolized MWCNT (10 mg/m^3, 5 hours per day; mass mode aerodynamic diameter 1.3 μm, count mode aerodynamic diameter 0.4 μm) for 2, 4, 8 and 12 days. All mice were sacrificed at one day post-exposure. Whole lung lavage (WLL) was conducted and polymorphonuclear leukocytes (PMNs) were assessed to index pulmonary inflammation. WLL fluid assays included lactate dehydrogenase (LDH) activity as a measure of cytotoxicity, albumin as a marker of the lung air-blood barrier integrity, as well as various cytokines. MWCNT lung burden ranged from 6.6 ± 0.5 μg/lung (2 days exposure) to 30.6 ± 1.2 μg/lung (12 days exposure). MWCNT-exposure induced increases in PMN levels from 152 ± 27 (x 10^3) PMNs/mouse (2 days exposure) to 1,893 ± 462 (x 10^3) PMNs/mouse (12 days exposure). MWCNT-exposure increased WLL fluid albumin from 0.18 ± 0.01 mg/ml (2 days exposure) to 0.41 ± 0.04 mg/ml (12 days exposure), while WLL fluid LDH increased from 131 ± 6 units/L (2 days exposure) to 286 ± 35 units/L (12 days exposure). MWCNT exposure caused increased interleukin-6 (IL-6), chemokine ligands 1 and 2 (CXCL1, CCL2), and granulocyte macrophage colony stimulating factor (GM-CSF) levels relative to air-exposed controls. In summary, these data indicate that exposure to aerosolized MWCNTs results in dose-dependent increases in pulmonary inflammation and damage, suggesting that aerosolized MWCNT may pose an occupational health hazard.

PL 54 BIODEGRADATION OF CARBON NANOTUBES BY EOSINOPHIL PEROXIDASE.

A. A. Kapralov[1], N. Yanamala[1], W. H. Feng[1], B. Fadeel[2], A. Star[3], A. A. Shvedova[4] and V. E. Kagan[1]. [1]*Center for Free Radical and Antioxidant Health, EOH, University of Pittsburgh, Pittsburgh, PA,* [2]*Division of Biochemical Toxicology, Karolinska Institutet, Stockholm, Sweden,* [3]*Department of Chemistry, University of Pittsburgh, Pittsburgh, PA and* [4]*Pathology and Physiology Research Branch, NIOSH, Morgantown, WV.*

Single-walled carbon nanotubes (SWCNT) having high potential for applications in nanotechnology, electronics and medicine can have adverse effects on human health and the environment. In vitro data indicate that SWCNT may be cytotoxic, largely by inducing oxidative stress. It was shown previously, that horseradish peroxidase and human myeloperoxidase (MPO) catalyse the biodegradation of SWCNT. Biodegradation of nanotubes by another mammalian peroxidase - eosinophil peroxidase (EPO) has not been studied. EPO exocytosed by eosinophils is actively involved in increased oxidant production during lung inflammation after treatment with pollutants and cigarette smoke. We found that incubation with EPO and H2O2 caused degradation of CNT over time, and the CNT suspension turned translucent. Neither EPO alone nor H2O2 alone caused nanotube degradation. Degragation of CNT was confirmed by: 1) visible-near-infrared spectroscopy showing decrease of characteristic metallic band (M1) and semiconducting (S2) transition band and 2) Raman spectroscopy demonstrating increase of disorder-induced D-band and decrease of tangential-mode G-band. Drastic changes in nanotube morphology were demonstrated by transmission electron microscopy. Biodegradation of CNT was higher in the presence of NaBr suggesting that not only reactive radical intermediates of EPO but also generated HOBr was

involved in the biodegradation process. Computer modeling was used to structurally characterize possible nanotube interaction sites with EPO. Studies are underway to assess oxidative biodegradation of CNT by EPO-rich activated human eosinophils. We conclude, that EPO can participate in enzymatic biodegradation of CNT after respiratory exposures during their production and handling. Supported by NIOSH OH008282; NIH NIAID U19 AI068021, HL70755, HL094488, EC-FP7-NANOMMUNE-214281

PL 55 LONG, FIBROUS CARBON NANOTUBES ACTIVATE THE NLRP3 INFLAMMASOME IN HUMAN MACROPHAGES AND INDUCE NEUTROPHILIA IN MICE LUNGS AFTER INTRATRACHEAL ADMINISTRATION.

J. Palomäki, E. M. Rossi, K. Savolainen, S. Matikainen and H. Alenius. *Finnish Institute of Occupational Health, Helsinki, Finland.*

Carbon nanotubes (CNT) are of great interest because of their multiple applications in industry but also because of their unknown health effects. Recent studies suggest that the high aspect ratio, a feature common with asbestosis, is a key factor for reported toxicity of certain CNT. The mechanism behind this phenomenon is, however, not known. In the present study, we studied whether different carbon nanomaterials are able to induce differences in pro-inflammatory reactions in human macrophages *in vitro*. Carbon black (Evonik Industries AG); short CNT (Baytubes C150HP); long, tangled CNT (CheapTubes Inc©); long, fibrous CNT (Mitsui&Co, Ltd) and crocidolite asbestos (PRC, South-Africa) were used for *in vitro* studies. We also exposed C57BL6 mice intratracheally to fibrous and tangled CNT to investigate their effects *in vivo*. Our results showed that only long, fibrous CNT and asbestos were able to induce robust IL-1β secretion from LPS-primed macrophages. The western blot (WB) analysis confirmed that the secreted IL-1β was biologically active. Ribonucleic acid interference-mediated gene knockdown experiments demonstrated cytoplasmic NLRP3 inflammasome is essential for fibrous CNT- and asbestos-induced IL-1β secretion. Moreover, we showed that CNT-induced NLRP3 inflammasome activation is dependent on P2X7 receptor and cathepsin B activity. *in vivo* experiments demonstrated that in contrast to tangled CNT, fibrous CNT exposure elicited prominent neutrophilia accompanied by the expression of neutrophil attracting chemokines confirming our *in vitro* findings. Taken together, our results demostrate that long, fibrous CNT have asbestos-like effects being clearly more hazardous than other CNT. Fibrous CNT activated NLRP3 inflammasome causing high production of pro-inflammatory cytokine IL-1β in human macrophages. In addition, fibrous CNT exposure induced significant neutrophilia in the mouse lungs *in vivo*. Further studies are needed to make reliable risk assessment of carbon nanotubes.

PL 56 PULMONARY FIBROTIC RESPONSE TO SUB-CHRONIC MULTI-WALLED CARBON NANOTUBE EXPOSURE.

R. R. Mercer, A. F. Hubbs, J. F. Scabilloni, L. Wang, L. A. Battelli, V. Castranova and D. W. Porter. *PPRB, NIOSH, Morgantown, WV.*

Multi-walled carbon nanotubes (MWCNTs) are manufactured carbon compounds with many commercial applications. To address the hypothesis that MWCNTs cause persistent pulmonary pathology, C57BL/6J mice were exposed by pharyngeal aspiration to 10, 20, 40 or 80 μg MWCNTs (mean dimensions of 3.9 μm x 49 nm) or vehicle. Lungs were preserved at 1, 7, 28 and 56 days post exposure to analyze the distribution of lung burden. Morphometric measurement of Sirius Red staining was used to assess the connective tissue response. At day 1 post-exposure 62.0±2.5 and 9.9±2 percent of the lung burden (mean±SE, N=7) were in alveolar macrophages and alveolar tissue, respectively. The remainder of the lung burden (18.0±3.2) was in the airways. By 56 days post-exposure, 68.7±3.9, 7.5±1.9 and 22.0±5.1 percent of MWCNT were in alveolar macrophages, alveolar tissue and granulomatous lesions, respectively. No MWCNTs were found in the airways at 56 days. At 56 days post-exposure the average thickness of connective tissue in alveolar regions was 0.11±0.01, 0.12±.01, 0.12 ±0.01, 0.16±0.01 and 0.19±0.01 μm (mean±SE, N=6) for vehicle, 10, 20, 40 and 80 μg dose groups, respectively. The connective tissue in the alveolar region demonstrated a progressive increase in thickness over time in the 80 μg exposure group (0.11±0.01, 0.14 ±0.01, 0.16±0.01 and 0.19±0.01 μm for 1, 7, 28 and 56 day). The distribution of lung burden was predominately within alveolar macrophages with approximately 8% delivery to the alveolar tissue. Despite the relatively low fraction of the lung burden being delivered to the alveolar tissue (7.5% at day 56), the average thickness of connective tissue in the alveolar region was increased over vehicle control by 45% in the 40 μg and 72% in 80 μg exposure groups. These results demonstrate that MWCNT have the potential to produce a progressive, fibrotic response in the alveolar tissues of the lungs.

PL 57 THE ROLE OF IL-1β SIGNALING IN NICKEL ASSOCIATED MULTI-WALLED CARBON NANOTUBE-INDUCED PULMONARY INFLAMMATION.

T. A. Girtsman. *CEHS, University of Montana, Missoula, MT.*

Exposure to certain engineered nanomaterials (ENM) has been associated with pathological changes in animal models raising concern that human health effects will emerge with increasing use. Some, but not all, ENM have been shown to activate the NLRP3 inflammasome. We have shown that nickel containing multi-walled carbon nanotubes MWCNT (Ni-MWCNT) can activate the NLRP3 inflammasome (NLRP3) in vitro using primary alveolar macrophages (AM) or THP-1 cells. Furthermore, we have also demonstrated NLRP3 activation in vitro correlates strongly with lung inflammation and pathology. Activation of caspase-1 via assembly of the NLRP3 inflammasome results in the conversion of pro-IL-1β to the active form of this proinflammatory cytokine (mature IL-1β), which is released by AM and is an important mediator of inflammation during infection. In this study, we investigated the role of IL-1β signaling to induce a pulmonary neutrophilic response using C57Bl/6 wild type or IL-1 receptor null mice (IL-1R-/-) after exposure to Ni-MWCNT. We found that Ni-MWCNT was effective in inducing pulmonary inflammation as indicated by neutrophilic influx and IL-1β secretion into the airways of wild type mice. The inflammatory response however, was abolished in mice deficient in the type I IL-1R, as indicated by significantly lower neutrophils in the inflammatory infiltrate. These data suggest an important role for IL-1β signaling in Ni-MWCNT-induced pulmonary inflammatory responses. This work was supported by NIH grants RC2-ES018742 and P20-RR017670.

PL 58 PULMONARY INFLAMMATION, EPITHELIAL HYPERPLASIA, AND LYMPH NODE TRANSLOCATION AFTER MULTI-WALLED CARBON NANOTUBE INHALATION.

A. Hubbs, V. Castranova, B. T. Chen, D. G. Frazer, W. McKinney, R. R. Mercer, M. L. Kashon, L. A. Battelli, P. Willard and D. W. Porter. *HELD, NIOSH, Morgantown, WV.*

Multi-walled carbon nanotubes (MWCNTs) are engineered nanotubes with multiple fullerene carbon walls, a high aspect ratio, and rapidly increasing industrial uses. To investigate the toxicity of inhaled MWCNTs, mice were exposed 5 hours/day to 10 mg/m^3 MWCNTs (Mitsui, MWNT-7, count mode aerodynamic diameter 420 nm) for 4, 8 or 12 days and sacrificed 24 h post-exposures. Histopathologic sections of lung and tracheobronchial lymph nodes were examined at all time points and sections of nose (4 levels) were examined after the 12 day exposure. In lung, the principal changes were 1) inflammation centered around the bronchioloalveolar junction, 2) vasculitis, and 3) bronchiolar epithelial hypertrophy and hyperplasia. These were seen in all exposed mice (n=8, 6 and 6 at 4, 8 and 12 days, respectively). Peribronchiolar inflammation was principally histiocytic and neutrophilic with occasional giant cells. In many macrophages, cytopathologic changes included 1) MWCNT penetration of the cytoplasmic membrane, 2) MWCNT penetration of nuclei, and 3) karyolysis. Vascular changes were present in all exposed mice but manifestations varied and included medial hypertrophy and contraction, mural neutrophil infiltrates, and rare mural MWCNTs. Bronchiolar hypertrophy and hyperplasia were present after 4 days and persisted. After 12 days of exposure, all mice had foci of peribronchiolar fibrosis and bronchiolar epithelial mucous metaplasia. Pleural MWCNTs were seen in two mice. In lungs of air exposed controls (n=8, 6 and 6 at 4, 8 and 12 days, respectively), vasculitis and bronchiolar changes were absent; a single focus of inflammation was seen in one mouse. MWCNT translocation to the tracheobronchial lymph node progressed with time and localized to the deep paracortex, the normal location of T lymphocytes and dendritic cells. In the nose, neutrophilic rhinitis and hyaline droplet formation were consistent changes. These findings suggest that chronic inhalation toxicity studies are needed.

PL 59 UNDERSTANDING CARBON NANOTUBE GENOTOXICITY.

L. M. Sargent[1], S. H. Reynolds[1], A. F. Hubbs[1], S. A. Benkovic[1], D. T. Lowry[1], M. L. Kashon[1], K. J. Siegrist[1], J. Mastovich[2], J. L. Sturgeon[2], K. L. Bunker[2] and C. Z. Dinu[3]. [1]*Toxicology and Molecular Biology, CDC/NIOSH, Morgantown, WV,* [2]*RJLeeGroup, Pittburgh, PA and* [3]*Chemical Engineering, West Virginia University, Morgantown, WV.*

Carbon nanotubes have many applications in medicine, electronics, aerospace and computer circuits. However, in order to use nanotubes for such applications, their potential genotoxic and cytotoxic effects need to be understood. We are studying nanotube interaction with cells and isolated cellular components, to determine

mechanisms responsible for cell fate. Specifically, we exposed primary and immortalized human epithelial cells to single- and multi-walled carbon nanotubes and examined the potential of nanotubes to induce genetic damage. The microscopy results showed fragmented centrosomes, multipolar mitotic spindles and errors in chromosome number following exposure to single walled carbon nanotubes (SWCNT). The larger multi-walled carbon nanotubes (MWCNT) primarily induced mitotic spindles with one mitotic spindle pole. The nanotubes associated with microtubules and centrosomes and formed biohybrids localized at the nucleus. In order to explain this behavior, we polymerized microtubules in vitro and we used kinesin motors to show integration with nanotubes and manipulation of functional biohybrid assemblies. In eukaryotic cells, microtubules play roles in intracellular transport as well as cell division. Microtubules assemble into mitotic spindles, while the kinesin motors are responsible for microtubule-based transport and cellular division. Our results demonstrate disruption of the mitotic spindle by nanotubes and give further evidence of the mechanism responsible for the disruption of cell division. These results suggest caution should be used in the handling and processing of carbon nanotubes.

PL 60 IMPACTS OF STRUCTURE AND FUNCTIONALIZATION ON TOXICOLOGICAL RESPONSE OF THE MODEL ORGANISM *DAPHNIA MAGNA.*

D. A. Arndt and R. Klaper. *School of Freshwater Sciences, University of Wisconsin Milwaukee, Milwaukee, WI.* Sponsor: R. Hutz.

Over the past decade there has been increasing economic investment in carbon nanotechnology resulting in an increased potential for the release of these particles to the environment, particularly to freshwater and marine systems. Previous studies have demonstrated the toxicity of these particles; however comparative data on these nanoparticles and the mechanism of their toxicity remains largely unknown. To resolve this, we examined how alterations of core structure and surface chemistry affect the interaction of a particle with the aquatic ecological, toxicological and genomic model species, *Daphnia magna.* Daphnia were exposed to carbon nanoparticles with differing core structures and functionalizations, and acute toxicity and chronic toxicity and reproduction were measured. In addition, global gene expression responses were used to determine the physiological impact at sub-lethal concentrations. High throughput sequencing was used to create libraries of genes with altered expression patterns for each nanomaterial. Life cycle toxicity analysis indicates a particle's core structure and surface functionalization have an influence on its relative toxicity. Sequencing analysis indicates that different genomic pathways are activated in the presence of an unfunctionalized fullerene (nC60) compared to a hydroxylated fullerene (C60-OH). This indicates that Daphnia differ in their physiological response to alterations in particle chemistry and structure. There is a potential to use these expression patterns to not only determine the genetic mechanism of a particle's toxicity, but also to use these molecular clues as biomarkers to determine environmental exposures to nanomaterials in aquatic environments.

PL 61 TOXICOGENOMICS EFFECTS OF WATER-SOLUBLE CARBON NANOTUBES.

D. A. Sarigiannis[1, 2], G. Cimino Reale[1], A. Collotta[1], R. Brustio[1], B. Casati[1], E. Roda[3], A. Profumo[6], D. Merli[6], E. Quartarone[5], P. Mustarelli[5], M. Fagnoni[5], T. Coccini[4], L. Manzo[3, 4] and E. Marafante[1]. [1]*Institute for Health and Consumer Protection, European Commission - Joint Research Centre, Ispra, Varese, Italy,* [2]*Chemical Engineering Department Aristotle University of Thessaloniki, Thessaloniki, Greece,* [3]*Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Pavia, Italy,* [4]*Toxicology Division, Salvatore Maugeri Foundation IRCCS, Pavia, Pavia, Italy,* [5]*Department of Physical Chemistry, University of Pavia, Pavia, Pavia, Italy and* [6]*Department of General Chemistry, University of Pavia, Pavia, Pavia, Italy.* Sponsor: L. Birnbaum.

The widespread use of functionalized carbon nanotubes (CNTs) makes the understanding of potential harmful effects highly important. Two cell culture systems, human A549 pneumocytes and HaCaT keratinocytes, were used to assess the modulation of gene expression due to exposure to single and multi-walled CNTs. Moreover, CD-1 male mice were exposed to the CNTs tested by intra-tracheal instillation and lung samples were taken and analyzed after 1 day of exposure. Differentially functionalized CNTs (MW-COOH and MW-NH2) were tested in comparison with pristine MWCNTs and SWCNTs. Toxicogenomic analysis included whole genome micro-array analysis and quantitative PCR using micro-fluidic cards for inflammation genes.
Comparison of gene expression between in vitro and in vivo exposure to NTs revealed differences in the level of biological response induced towards oxidative stress, inflammation and apoptosis. Differential modulation in gene expression after in vivo exposure was observed as a function of single or multiple wall geometry and presence of specific functional groups. MW-COOH showed a very high degree of up-modulation of the genes coding for chemokine ligands clinically associated with the onset of lung fibrosis in humans. This effect was much less pronounced with MW-NH2 or SWCNT, whereas pristine MWVNT did not show any statistically significant modulation in gene expression. The main biological pathways induced by the tested CNTs were chemokine and cytokine induced inflammation and oxidative stress. This study indicates that CNT functionalization modulates the advent of early biological events affecting their health effects.

PL 62 DECIPHERING MECHANISMS UNDERLYING PROLONGED MALE INFERTILITY FOLLOWING A CLINICALLY-RELEVANT MULTI-CYCLE CISPLATIN TREATMENT.

J. Harman[1, 2] and J. Richburg[1, 2]. [1]*College of Pharmacy, Division of Pharmacology/Toxicology, University of Texas at Austin, Austin, TX and* [2]*Center for Molecular and Cellular Toxicology, University of Texas at Austin, Austin, TX.*

Cisplatin is a chemotherapeutic compound that initiates apoptosis. A typical clinical regimen consists of repeated cycles of 5-7 daily injections of low dose cisplatin with a 1-2 week recovery period. An unfortunate side effect of cisplatin exposure in males is a prolonged, sometimes permanent, infertility. Previously, we developed a clinically-relevant treatment paradigm in adult C57/Bl/6J mice (repeated cycles of 2.5mg/kg/day for 5 days followed by a 7 day recovery period) and discovered that the severity of testicular damage is more dependent on the number of cycles of treatment than the cumulative dose. Theoretically, spermatogonial stem cells (SSCs) should be able to repopulate the testis after cisplatin exposure has ended. We hypothesize that an increase in the mitotic activity of SSCs during the initial exposure to cisplatin renders them increasingly susceptible to induced injury during the next treatment cycle and underlies the mechanism of treatment-induced infertility. Here we investigate Sertoli cell (SC) factor(s) that stimulate SSCs to exit quiescence and enter the cell cycle after cisplatin exposure. IHC analysis of BrdU incorporation after one cycle of cisplatin exposure showed a 13-fold increase in the proliferative rate of early germ cells over controls. Glial cell line-derived neurotrophic factor (GDNF) is secreted by SCs, and has been implicated in SSC regulation. Analysis of testicular cross sections revealed an increase in GDNF protein immunostaining in treated mice that was particularly prominent along the basal membrane, the region where SSCs reside. Taken together, these preliminary data provide evidence that an increased secretion of GDNF from SCs occurs at a time that correlates with an increased proliferative rate of SSCs after cisplatin-induced testicular injury. Future experiments are targeted to test the direct role of GDNF in increasing the sensitivity of SSCs to cisplatin-induced injury.

PL 63 DRAMATIC STRAIN DIFFERENCES IN SENSITIVITY OF RAT SPERMATOGENESIS TO IRRADIATION AND OTHER GONADAL TOXICANTS.

M. Meistrich, C. Weng and M. AbuElhija. *Experimental Radiation Oncology, MD Anderson Cancer Center, Houston, TX.*

We previously reported (J Androl 14:257,1993) dramatic differences in the recovery of spermatogenesis in rat testes from the chemotherapy drug procarbazine; Lewis and Lewis-Brown Norway (BN) F1 hybrids were uniformly sensitive and outbred Sprague-Dawley (SD) rats were resistant but with wide variations, suggesting genetic differences. To systematically investigate these differences, we measured the recovery of spermatogenesis after irradiation in 5 inbred strains (BN, Lewis, Fischer 344, Wistar Kyoto, and SHR, which is derived from Wistar) and 2 outbred stocks (Long-Evans and Sprague-Dawley [SD]). Rat testes were irradiated with 5 Gy and tissue harvested 10 weeks later for testicular sperm counts and histological analysis, particularly the tubule differentiation index, TDI, which is the percentage of tubules containing differentiated cells. Lewis and BN rats were by far the most sensitive, Long-Evans, Wistar, Fischer, and SHR (listed in order of increasing recovery) were more resistant, and SD had the highest spermatogenic recovery. For example, testis weights were decreased to 23%, 29%, and 35% of control in BN, Wistar, and SD rats respectively. Sperm counts were decreased to 0.005×10^6, 4×10^6, and 19×10^6 for BN, Wistar, and SD, respectively, compared to about 200×10^6 for controls. By histology, the BN testes showed atrophy with almost complete germ cell loss while the SD appeared relatively normal, with only a reduction in the fractions of tubules with spermatids; TDIs were 1%, 57% and 98% in BN, Wistar, and SD, respectively. The atrophic tubules in all rats showed the presence of type A stem spermatogonia, indicating that the failure of recovery was not due to the loss of

stem cells but rather a treatment induced block in the ability of the spermatogonia to differentiate, which is also observed after exposure to hexanedione, DBCP, and other chemicals (J Androl 24:135,2003). It is important to elucidate the mechanism of sensitivity so that, in toxicology testing, a strain be used with a similar mechanism of sensitivity as observed in human.

PL 64 THE EFFECTS OF NEONATAL EXPOSURE TO DECA-BROMINATED DIPHENYL ETHER ON APICAL ECTOPLASMIC SPECIALIZATION IN MOUSE TESTIS.

H. Miyaso[1], Y. Matsuno[1, 2], M. Komiyama[1], S. Ochiai[1], Y. Igoshi[1] and C. Mori[1, 2]. [1]*Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan and* [2]*Center for Preventive Medical Science, Chiba University, Chiba, Japan.*

Polybrominated diphenyl ethers (PBDEs) are additive flame-retardants and deca-brominated diphenyl ether (deca-BDE), one of the PBDEs, has been particularly used worldwide. On the other hand, some recent studies have reported that deca-BDE can cause adverse health effects on humans and animals. Other studies have reported that deca-BDE is an endocrine disruptor. Endocrine disruptors have been reported to be targeted to male reproductive system (e.g., the testes), and that exposure to endocrine disruptor during prenatal/neonatal period causes more adverse effects than that in adults. However, few studies have reported the effects of neonatal deca-BDE exposure on the male reproductive system. In this study, we examined the effect of neonatal deca-BDE exposure on mouse testes. Administration of deca-BDE dissolved in corn oil was performed by subcutaneous injection during neonatal 1-5 days, and vehicle only is injected to control group. Testes were collected in 12-weeks-old, and evaluated by histological examination and Western blot analysis. Our results demonstrate that 1) the weight of the testes was decreased with neonatal deca-BDE exposure, 2) exposure to deca-BDE caused a decrease in the number of Sertoli cells and spermatogenic cells, in particular elongated spermatids, 3) cortactin and mitogen-activated protein kinase (MAPK) p44/42, which are proteins related to apical ectoplasmic specialization, had higher phosphorylation levels in the deca-BDE exposure group of mice as compared to the control group. These results suggest the possibility that neonatal deca-BDE exposure causes a decrease in the number of Sertoli cells, which contributes to a functional disruption of apical ectoplasmic specialization that affects the interaction between elongated spermatids and Sertoli cells and causes a decrease in the number of elongated spermatids. Thus neonatal deca-BDE exposure might have adverse effects in mouse testes and spermatogenesis.

PL 65 GENE EXPRESSION CHANGES IN THE TESTIS FOLLOWING X-RADIATION AND 2, 5-HEXANEDIONE EXPOSURE.

N. Catlin, S. N. Campion and K. Boekelheide. *Brown University, Providence, RI.*

The testis is a complex reproductive organ composed of several different cell types, which interact in a paracrine manner to carry out spermatogenesis. Reproductive toxicants can target one of the many cell types in the testis, including Sertoli cells and germ cells. X-radiation (x-ray) directly induces germ cell apoptosis; however, this apoptotic response is attenuated by co-exposure to the Sertoli cell toxicant, 2,5-hexanedione (HD). Previous global gene expression analysis identified individual apoptosis-related genes that were significantly up-regulated after exposure to either HD or x-ray alone (Egr1, Angptl4, Bbc3, Casp3, AEN). These genes were used as probes to identify threshold doses for induction of gene alterations in a low-dose extrapolation study, using qRT-PCR and microarrays. In this study, Fischer rats were dosed with either HD (0.125%, 0.2% or 0.33%) for 18 days and sacrificed on day 18, or with x-ray (0.5Gy or 0.1Gy) with sacrifice occurring 3 hours after exposure. For the above genes, we saw a dose dependent increase in expression for all genes; with the exception of Egr1. Microarray analysis was performed on the individual toxicants (HD or x-ray), using the same tissues utilized for the qRT-PCR studies. Preliminary analysis of these array results reveals that we were able to detect gene alterations at these low doses, illustrated with clustering of biological replicates. A total of 58 and 408 overlapping genes were identified between the treatment groups for HD (0.125%, 0.2% and 0.33%) and x-ray treatment groups (0.1Gy and 0.5Gy), respectively. Having investigated the apoptotic gene responses to low dose x-ray exposure alone, rats will be co-exposed to x-ray and HD in the low dose range and gene alterations will be measured by microarray and real time PCR. We hypothesize that low-dose extrapolation of 2,5-hexanedione and x-radiation co-exposure will distinguish adaptive from adverse gene alterations and further elucidate the mechanism(s) underlying the phenotypic consequences of co-exposure.

PL 66 C/EBP AND C-MYC COOPERATE FOR THE TRANSCRIPTIONAL SUPPRESSION OF SERTOLI CELL TIMP-2 IN RODENTS FOLLOWING MEHP EXPOSURE.

P. Yao, Y. Lin and J. H. Richburg. *Center for Molecular and Cellular Toxicology, Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.*

Investigating the molecular and cellular basis of toxicant-induced testicular malfunction can be informative to clinicians for determining biomarkers and new diagnostic methods as well as identifying effective targets for fertility therapies. Our previous studies show that the prototypical toxic phthalate monoester, mono-2-(ethylhexyl) phthalate (MEHP), suppresses Sertoli cell TIMP-2 levels and consequently activates MMP-2 in the seminiferous tubule which is important for triggering germ cell apoptosis and germ cell detachment. The balance of TIMP-2 and MMP-2 appears to be critical in controlling homeostasis in the testis. The addition of TIMP-2 in vitro significantly suppressed MEHP-induced germ cell sloughing, indicating that TIMP-2 plays a primary role in this process. These novel findings lead us to further examine the mechanisms that account for the decrease in Sertoli cell TIMP-2 following MEHP exposure. In this current study, we characterize the transcriptional regulation of TIMP-2 gene in primary rat Sertoli cells after MEHP exposure. Sequential deletion of the TIMP-2 5'-upstream activating sequence (1200 bp) was used to determine transcriptional activities in response to MEHP. Our results indicate that C/EBP-α and c-Myc may participate in the transcriptional suppression of testis TIMP-2 following MEHP exposure. The addition of follicle-stimulating hormone (FSH) rescues MEHP-suppressed TIMP-2 levels and causes the translocation of CREB and C/EBP-α into the nucleus. The down-regulation of TIMP-2 by MEHP exposure is blocked by adding forskolin (a cAMP-elevating agent) in a dose-dependent manner in vitro. These observations suggest that the decrease in Sertoli cell TIMP-2 by MEHP exposure is cAMP-dependent. Taken together, the participation of FSH-stimulated cAMP in controlling TIMP-2 expression is likely suggested to be the consequence of decreased Sertoli cell support in response to MEHP exposure.

PL 67 *IN UTERO* DIBUTYL PHTHALATE EXPOSURE CONCOMITANTLY INHIBITS FETAL TESTIS STEROIDOGENIC AND SREBP2-DEPENDENT CHOLESTEROLOGENIC PATHWAYS.

K. Johnson[1], M. Viereck[1], E. McDowell[1] and Q. Xia[2]. [1]*Biomedical Research, Alfred I. duPont Hospital for Children, Wilmington, DE and* [2]*National Institutes of Statistical Sciences, Research Triangle Park, NC.*

Fetal rat phthalate exposure produces a spectrum of male reproductive tract malformations downstream of reduced Leydig cell testosterone production, but the molecular mechanism of phthalate perturbation of Leydig cell function is not well understood. Using bioinformatics to examine several fetal testis gene expression microarray datasets from dibutyl phthalate (DBP) exposed susceptible (rat) and non-susceptible (mouse) rodents, decreased expression of metabolic pathways governing carbohydrate, amino acid, and energy metabolism were observed in both rats and mice; however, fatty acid, steroid, and cholesterol synthesis pathways and gene sets induced by the master lipid metabolism transcriptional regulator sterol regulatory binding protein (SREBP) were inhibited in rats but induced in mice. In rats exposed to 100 or 500 mg/kg DBP from gestational days (GD) 16 to 20, a correlation was observed between GD20 testis steroidogenic inhibition and reductions of testis cholesterol synthesis endpoints including testis total cholesterol levels, Srebf2 gene expression, and cholesterol synthesis pathway gene expression. SREBP2 immunoexpression was detected in all fetal rat testis cells but was highest in Leydig cells. Quantification of SREBP2 immunostaining showed that 500 mg/kg DBP exposure significantly reduced SREBP2 immunoexpression in rat fetal Leydig cells but not in seminiferous cords. By Western analysis following 500 mg/kg DBP exposure, there was a trend toward reduced total testis SREBP2 expression ($p < 0.08$). Together, these data suggest phthalate-induced inhibition of fetal testis steroidogenesis is closely associated with reduced activity of lipid metabolism pathways and Leydig cell SREBP2-dependent cholesterologenesis.

PL 68 ACRYLAMIDE AND GLYCIDAMIDE ARE MUTAGENIC IN SPERMATOGONIAL STEM CELLS OF BIG BLUE MICE.

N. Mei[1], R. Wang[1, 3], L. P. McDaniel[1], S. D. Shelton[1], D. R. Doerge[2] and M. G. Manjanatha[1]. [1]*Division of Genetic and Molecular Toxicology, NCTR/FDA, Jefferson, AR,* [2]*Division of Biochemical Toxicology, NCTR/FDA, Jefferson, AR and* [3]*Division of Health Effects Research, Japan National Institute of Occupational Safety and Health, Kawasaki, Japan.*

Acrylamide (AA) is an industrial chemical, a by-product of fried starchy foods, and a mutagen and rodent carcinogen. It can also cause damage during spermatogenesis. In this study, we investigated whether AA and its metabolite glycidamide (GA)

induce mutagenic effects in the germ cells of male mice. Male Big Blue transgenic mice were administered 1.4 or 7.0 mM of AA or GA in the drinking water for up to 4 weeks. Testicular cII mutant frequency (MF) was determined 3 weeks after the last treatment, and the types of the mutations in the cII gene were analyzed by DNA sequencing. The testes cII MFs in mice treated with either the low or high exposure concentrations of AA and GA were increased significantly. There was no significant difference in the cII MFs between AA and GA at the low exposure concentration. The mutation spectra in mice treated with AA (1.4 mM) or GA (both 1.4 and 7.0 mM) differed significantly from those of controls, but there were no significant differences in mutation patterns between AA and GA treatments. Comparison of the mutation spectra between testes and livers showed that the spectra differed significantly between the two tissues following treatment with AA or GA, whereas the mutation spectra in the two tissues from control mice were similar. These results suggest that AA possesses mutagenic effects on testes by virtue of its metabolism to GA, possibly targeting spermatogonial stem cells, but possibly via different pathways when compared mutations in liver.

PL 69 1, 3-DINITROBENZENE: REVISITING THE POSTULATED MODE OF ACTION FOR ITS TESTICULAR TOXICITY.

S. Ludwig, H. Tinwell, D. Rouquié and R. Bars. *Research Toxicology, Bayer SAS, Sophia Antipolis Cedex, France.*

Many nitroaromatic compounds used to manufacture polymers, pesticides and dyes are known testicular toxicants. One such compound, 1,3-dinitrobenzene (DNB), causes lesions in the rat testis, decreased sperm number, and abnormal sperm morphology and motility. As ultrastructural changes were found in the Sertoli cells before changes in germ cell morphology, the Sertoli cell has been postulated as the primary target for the toxic action of DNB. We have conducted studies to better understand the pathogenesis of the testicular effects produced by DNB, and to investigate its mode of action. Rats were orally exposed to 0.1-4 mg DNB/kg/day for 4 days, and the resultant effects were investigated using standard parameters (organ weight, histopathology, testosterone measurements) and molecular tools (microarray analyses and qPCR). Testosterone concentrations were not affected at any dose level, but marked histopathological lesions were recorded in the testes at 4mg/kg/day. Global transcriptomic analysis of rat testes revealed hundreds of genes differentially expressed following exposure to 4mg/kg/day. The major biological processes affected were related to cell cycle and cell death. In particular, we identified an alteration in the expression of genes associated with cell cycle progression ("mitotic roles of polo-like kinase"). Our molecular data (microarray and qPCR), can be correlated with our histopathological data and also with earlier publications (particularly for apoptosis). These observations have been further investigated in a second study, where rats were sacrificed at 8, 24, 48 and 72 hours after a single oral dose of 4mg/kg DNB. The preliminary molecular data indicate alterations of common biological processes induced by DNB after a single or after 4 doses. Surprisingly, in contrast to the 4 day data, plasma testosterone was reduced 48h after a single dose, which was associated with a down-regulation of genes involved in steroidogenesis. As Sertoli cells and not Leydig cells have been previously proposed as the primary target for DNB, this observation warrants further investigation.

PL 70 ITCH PROMOTES MEHP-INDUCED GERM CELL APOPTOSIS.

J. L. Dwyer[1], Y. Lin[2], P. Yao[2] and J. H. Richburg[2, 1]. [1]*Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX and* [2]*Center for Molecular and Cellular Toxicology, University of Texas at Austin, Austin, TX.*

Spermatogenesis is an intricate process that depends on Sertoli cell (SC) support for germ cell (GC) development. Exposure to various environmental toxicants can injure SCs and disrupt this process. Di-ethylhexyl phthalate (DEHP) is used to impart flexibility during the manufacturing of plastic products, but it leaches out and is often found throughout the environment. Once in the body, DEHP is rapidly hydrolyzed to mono-ethylhexyl phthalate (MEHP), a well-described SC toxicant. Previous studies from our lab revealed that MEHP causes an increase in the expression of SC-derived FasL, a ligand belonging to the Tumor Necrosis Family of proteins involved in triggering apoptosis. It is proposed that the increased levels of FasL after MEHP treatment allows for activation of its receptor on GCs, Fas, triggering a cascade of caspase cleavage and ultimately apoptosis. The cellular FLICE-Like Inhibitory Protein (c-FLIP) is also present in GCs, and has been shown to inhibit apoptosis by preventing caspase-8 cleavage. An important negative regulator of c-FLIP is the E3 ubiquitin ligase Itch, which targets c-FLIP for proteasomal degradation. In order to further study the regulation of c-FLIP and its influence on MEHP-induced GC apoptosis, Itch knockout mice were evaluated. Histological examination of peripubertal mice revealed that they are structurally similar to their wild type C57/BL6J counterparts. However, an analysis of the number of mature spermatids in the testis of Itch knockout mice revealed a significant decrease in their production as compared to C57 mice. Following MEHP exposure, C57 mice show an increase in GC apoptosis, while the Itch knockout mice appear to be partially protected from injury. Protein analysis revealed that Itch mice have higher levels of c-FLIP, and these levels slightly increase following MEHP exposure. Taken together, these data suggest that c-FLIP and Itch may play an important role during GC development and in modulating the response to MEHP exposure.

PL 71 COMPARISON OF THE EMBRYONIC STEM CELL TEST, THE WHOLE EMBRYO CULTURE, AND THE ZEBRAFISH EMBRYO TEST AS ALTERNATIVE METHODS FOR DEVELOPMENTAL TOXICITY TESTING OF TRIAZOLES.

E. de Jong[1, 2], M. Barenys[3], S. Hermsen[2, 4], A. Verhoef[2] and A. H. Piersma[1, 2]. [1]*Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands,* [2]*National Institute for Public Health and the Environment, Bilthoven, Netherlands,* [3]*Toxicology Unit, Public Health Department, School of Pharmacy, University of Barcelona, Barcelona, Spain and* [4]*Department of Health Risk Analysis and Toxicology, NUTRIM, University of Maastricht, Maastricht, Netherlands.* Sponsor: H. van Loveren.

The high experimental animal use in developmental toxicity testing has stimulated the search for less animal intensive alternatives. Three widely studied alternative assays are the mouse embryonic stem cell test (EST), the zebrafish embryotoxicity test (ZET) and the postimplantation rat whole embryo culture (WEC). The goal of this study was to determine their efficacy in predicting the relative developmental toxicity of six triazole compounds, flusilazole, hexaconazole, cyproconazole, triadimefon, myclobutanil and triticonazole. Triazoles are antifungal agents used in agriculture and medicine. They are known to induce craniofacial and limb abnormalities in rodents. We determined the correlation between the effects of the compounds in the alternative assays and their in vivo developmental toxicity. Both the ZET and the WEC showed a general pattern of teratogenic effects after exposure to the triazoles, mainly consisting of effects on the branchial arches in the WEC and head and heart anomalies in the ZET, which corresponds to the type of abnormalities found in vivo. In the EST all triazole compounds inhibited cardiomyocyte differentiation. In the alternative assays, effects observed were concentration-dependent, allowing potency ranking and comparison with in vivo ranking. Overall, the ZET gave the best prediction of the relative developmental toxicity of the tested compounds, followed by EST and WEC, respectively. These results may be explained by differences in compound kinetics, developmental stage and complexity between alternative assays.

PL 72 PREDICTING DEVELOPMENTAL TOXICITY OF TOXCAST™ PHASE I CHEMICALS USING HUMAN EMBRYONIC STEM CELLS AND METABOLOMICS.

N. C. Kleinstreuer[2], P. R. West[1], A. M. Weir-Hauptman[1], A. M. Smith[1], T. B. Knudsen[2], E. L. Donley[1] and G. G. Cezar[1, 3]. [1]*Stemina Biomarker Discovery, Inc., Madison, WI,* [2]*ORD/NCCT, U.S. EPA, Durham, NC and* [3]*University of Wisconsin-Madison, Madison, WI.*

EPA's ToxRefDB contains prenatal guideline study data from rats and rabbits for over 240 chemicals that overlap with the ToxCast in vitro high throughput screening project. A subset of these compounds were tested in Stemina Biomarker Discovery's developmental toxicity platform, an in vitro method combining human embryonic stem (hES) cells and metabolomics to discover biomarkers of developmental toxicity. The purpose of this pilot study was to perform LC-MS based non-targeted metabolomic analysis on the supernatant of hES cell cultures dosed with blinded EPA test chemicals to identify human metabolites subject to chemically induced alterations and biochemical signatures which may be indicative of potential human developmental toxicity. Significant fold changes in endogenous human metabolites were detected for 83 annotated mass features in response to the subset of ToxCast chemicals. The annotations were mapped to specific human metabolic pathways with nicotinate and nicotinamide metabolism, pantothenate and CoA biosynthesis, glutathione metabolism, and arginine and proline metabolism pathways most affected. Stemina's predictive DevTox® model, trained on 22 pharmaceutical agents of known teratogenicity and differing potency, was applied to the blinded EPA test compound data to test predictivity for mammalian in vivo data. The model correctly predicted teratogenicity for eight of eleven compounds and an

additional compound showed agreement with animal data at the low treatment dose. Thus, our initial results indicate this platform as an alternative to animal models for predicting developmental toxicity and providing mechanistic information about the underlying biochemical pathways. [This abstract does not necessarily reflect U.S. EPA policy]

PL 73 TESTING OF PRO-TERATOGENS IN THE EMBRYONIC STEM CELL TEST: SIMULATION OF METABOLIC CONVERSION BY TREATMENT WITH DEFINED MIXTURES OF PARENT COMPOUNDS AND THEIR RESPECTIVE METABOLITES.

A. E. Seiler, J. Kaltenhaeuser, U. Gruber and A. Luch. *ZEBET, Federal Institute for Risk Assessment, Berlin, Germany.* Sponsor: R. Chapin.

Lack of metabolic activity is one of the drawbacks of many in vitro assays. It still represents a major challenge to supplement existing in vitro methods with biotransformation systems. To estimate the amount of parent compound required of being converted into its major metabolite before affecting the outcome in the validated embryonic stem cell test (EST) we mimicked bioactivation by testing fix combinations of selected chemicals with their respective teratogenic metabolite. Effects of parent compound/metabolite pairs were assessed separately and in combination at ratios of 80:20 and 50:50, thus mimicking incomplete bioactivation in the range of 20 and 50%, respectively. Valpromide (VPD), retinol (ROH) and albendazole (ABZ) were chosen as parent compounds together with their metabolites, i.e. valproic acid (VPA), all-trans retinoic acid (RA) and albendazole sulfoxide (ASO), respectively, and all were tested for their effects on mESC differentiation and cytotoxicity. The active metabolite VPA was 12-times more potent to inhibit differentiation into contracting cardiomyocytes compared to its parent, VPD, while no such significant differences were detected for half-maximal cytotoxic concentrations (IC50). Comparable inhibition of differentiation occurred in the 50:50 and 80:20 mixtures at calculated VPA concentrations of about 200-430 μM, thus indicating saturation of the developmental block in culture already at 20% conversion of the pretoxin. RA was 200- to 300-fold more potent in inhibiting cardiomyocyte differentiation and 700-fold more potent in reducing cell viability when compared to its mother compound, ROH. ABZ was more toxic (factor 40) in comparison to its metabolite ASO. Inhibition of differentiation as well as cytotoxicity could be associated with an ABZ concentration of about 0.2 – 0.4 μM. The EST, which itself lacks any metabolic competence, can be used as readout for assessing developmental toxicity of fixed pairs of compounds and its respective metabolites.

PL 74 COREXIT EC9527A INHIBITS RETINOL SIGNALING IN P19 MOUSE PLURIPOTENT CELLS.

Y. Chen and D. H. Reese. *DMB, OARSA, CFSAN, U.S. FDA, Laurel, MD.* Sponsor: S. Sahu.

The oil dispersants Corexit EC9500A and Corexit EC9527A were used to remediate the effects of the 2010 Deepwater Horizon oil spill in the Gulf of Mexico. Early toxicity testing indicated that Corexit EC9527 was more toxic to various marine organisms and was subsequently replaced by the less toxic, Corexit EC9500A. The effects of Corexit dispersants on cells of higher organisms are largely unknown, however. In this study, we evaluated their effects in a newly developed assay that detects chemicals that disrupt the metabolism of vitamin A (retinol) to its biologically active metabolite, retinoic acid (RA). RA regulates the expression of key developmental genes, such as the Hox genes and is essential for normal embryonic development and maintenance of cellular phenotype in adults. The assay uses the P19 mouse pluripotent embryonal carcinoma cell that metabolizes retinol to RA and can be induced to differentiate into neurons by RA. We found that Corexit EC9500A was considerably more toxic to P19 cells than Corexit EC9527A using the MTT assay and therefore used Corexit EC9527A in these studies. At non-toxic concentrations, the dispersant significantly inhibited the ability of retinol to induce the expression of Hoxa1. During the synthesis of RA, retinol is first converted to retinaldehyde (RAL) which is then converted to RA; retinaldehyde also upregulates Hoxa1, after conversion to RA. To determine whether Corexit EC9527A interferes with the synthesis of RA or acts at the level of gene transcription, we measured its effects on the ability of all three compounds to induce Hoxa1. Corexit EC9527A inhibited induction by retinol and RAL but not by RA indicating that the inhibitory action of the dispersant is at the level of RA synthesis from retinol. It is unknown at this point which component(s) of Corexit EC9527A are inhibitory. Studies are ongoing including experiments to determine if the dispersant affects the ability of the P19 cell to differentiate into neurons.

PL 75 TERATOGENICITY OF VALPROIC ACID DERIVATIVES: EVALUATION OF STRUCTURE-ACTIVITY RELATIONSHIPS USING THE EMBRYONIC STEM CELL TEST.

C. Riebeling[1], A. E. Seiler[1], K. Becker[2], R. Buesen[1, 6], D. Eickel[5], J. Kaltenhaeuser[1], F. Meyer[3], H. Nau[4], R. Pirow[1], B. Slawik[1], A. Visan[1], J. Volland[3], H. Spielmann[1] and A. Luch[1]. *[1]ZEBET, Federal Institute for Risk Assessment, Berlin, Germany, [2]Nonclinical Drug Safety, Bayer Schering Pharmacology AG, Berlin, Germany, [3]Nycomed GmbH, Barsbüttel, Germany, [4]Department of Food Toxicology and Chemical Analysis, University of Veterinary Medicine Hannover, Hannover, Germany, [5]Product Application Laboratory, Advion BioSystems, Ithaca, NJ and [6]Mechanistic Toxicology, BASF, Ludwigshafen, Germany.* Sponsor: R. Chapin.

The embryonic stem cell test (EST) which is based on the morphometric measurement of cardiomyocyte differentiation and cytotoxicity parameters represents a validated method for the detection and classification of compounds according to their teratogenic potency. However, more work is required to assess its applicability domain and to improve its predictive capacity before gaining regulatory acceptance. We picked valproic acid (VPA) as a model compound to evaluate the suitability of the EST for distinguishing between developmental toxicity potencies of substances with closely related structures. Here we investigated six closely related analogues of VPA whose teratogenic potential has been previously determined in the NMRI exencephaly mouse model. Determining the concentration of VPA and of each of its six derivatives at which half maximal inhibition of differentiation occurs in the EST revealed a similar ranking as found previously. Distinct embryotoxicities in vivo of stereoisomers which differ only in their spatial configuration were reproduced by the EST. Similarly, an increased potency in vivo correlating with longer chain length of the congener was evident as higher toxicity in the EST. Our data demonstrate that the EST is capable of differentiating among closely related molecules according to their embryotoxic potency. As toxicological endpoints, both differentiation and cytotoxicity in vitro have to be considered to assess teratogenicity comparable to in vivo results. In conclusion, all substances were ranked in the same order and in full accordance to data obtained in the NMRI exencephaly mouse model.

PL 76 THE ROLES OF MKK4 AND MKK7 IN DEVELOPMENTAL TOXICITY.

J. Wang[1, 2], L. Chen[1], L. Zhang[2] and Y. Xia[1]. *[1]Environmental Health, University of Cincinnati, Cincinnati, OH and [2]Histology & Embryology, Southern Medical University, Guangzhou, China.*

The Mitogen-Activated Protein Kinases (MAPKs) are responsible for relaying extracellular signals to cellular responses. In mammals, there are three major MAPK groups, the extracellular signal regulated kinases (ERKs), the JUN-N-terminal kinases (JNKs) and the p38s, all activated through a MAP3K-MAP2K-MAPK cascade. *In vivo* gene knockout studies suggest the involvement of the MAPK cascades in early embryonic development, but whether the MAPKs are involved in developmental toxicity of environmental exposure is not well understood. The MKK4 and MKK7 are the MAP2Ks responsible for activation of the JNKs and p38s, known as stress activated protein kinases sensitive to environmental agents. We hypothesize that some environmental agents may act through MKK4 and MKK7 to affect JNK and p38 thereby perturbing developmental programs and exerting developmental toxicity. We used wild type, MKK4- and MKK7-knockout mouse embryonic stem cells (ESCs) and *in vitro* ESC differentiation to investigate lineage specification in early developmental stages. We showed that MKK4 and MKK7 were dispensable for ESC self-renew and pluripotency, but had unique signaling and functional properties in differentiation. While MKK4 and MKK7 both contributed to activation of the JNK-c-JUN pathways and survival of differentiated cells, MKK4 was also required for activation of the p38-ATF2 cascades to promote cardiogenesis. Exposure to non-cytotoxic doses of environmental toxic metals, such as cadmium (Cd) and chromium (Cr), significantly suppressed cardiac differentiation. Moreover, cardiac toxicity of Cr, but not Cd, was largely prevented in MKK7-null cells, suggesting that Cr toxicity was mediated in part through MKK7. Based on these data we suggest that the MAPKs may be activated by physiological and environmental stimuli and in turn regulate early embryonic development and lineage specification. The molecular and signaling mechanisms of MAPKs in developmental toxicity can be conveniently evaluated *in vitro* using ESC culture.

PL 77 DEVELOPMENT OF EMBRYONIC STEM CELL-DERIVED NEURONS FOR BOTULINUM NEUROTOXIN RESEARCH AND DRUG DISCOVERY.

P. McNutt and M. Mesngon. *USAMRICD, Gunpowder, MD.* Sponsor: G. Rockwood.

Identification of effective small molecule inhibitors of botulinum neurotoxins (BoNT) have not been successful, in large part due to the lack of a neuromimetic cell-based platform. We report that neurons differentiated from embryonic stem cells are compatible with drug discovery methodologies and suitable for molecular characterization of intoxication. Suspension-adapted ESCs reliably differentiate into homogenous neuron cultures (ESNs) that express BoNT substrate proteins, exhibit action potentials, construct synapses and undergo trans-synaptic signaling. ESNs display characteristic synaptic architectures, and extracellular electrophysiology using multielectrode arrays (MEAs) verifies the presence of action potentials and network activity. Exposure of ESNs to BoNT/A and /E results in cleavage of target SNARE proteins with EC50s of 0.8 and 67.4 pM, respectively. These sensitivities are equivalent to primary fetal spinal cord neurons and two-to-three orders of magnitude improved over alternative cell-based model systems, suggesting that ESNs internalize and process BoNTs similar to primary neurons. Exposure of ESNs to 10xEC50s of BoNT/A between 5-90 days after plating causes complete SNAP25 cleavage and inhibition of neurotransmitter release. The ability to generate post-mitotic neurons via cell culture allows application of novel research methodologies to BoNT drug discovery. For example, we are using systems biology approaches to identify novel therapeutic targets for BoNT research. Candidate targets will be interrogated by multiple methods, including neuronal expression of genetically encoded reporters and knock-down of endogenous gene expression. We are determining differentiation conditions that enrich for cholinergic motor neurons in order to generate neuromuscular junctions in vitro for increased physiologic relevancy. Finally, we are evaluating the use of multielectrode arrays as a network-based BoNT drug discovery program. We will discuss the utility of ESNs as a cell-based in vitro toxicology platform for BoNT therapeutics as well as for other neuron-specific insults.

PL 78 TCDD-INDUCED MODIFICATION OF TRANSCRIPTION FACTOR SIGNALING AND CARTILAGE DYSMORPHOGENESIS *IN VIVO* .

W. Dong[1, 2] and S. W. Kullman[1]. [1]*Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC and* [2]*College of Animal Science and Technology, Inner Mongolia University for the Nationalities, Tongliao, Inner Mongolia, China.*

Mesenchymal stem cells (MSC) are highly regulated multipotent stem cells that give rise to multiple cell and tissue types including cartilage and bone. Formation of cartilage consists of a highly coordinated and orchestrated series of events involving: commitment and differentiation of mesenchymal cells to chondrocytes, programmed and structured maturation and eventual formation of bone surrounding the perichondral structures. Our laboratory has identified a novel hypural cartilage phenotype in medaka that serves as a prototypic focus of cartilage dysmorphogenesis following 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) exposure. Here we establish the localization and differential gene expression for key regulatory factors associated with MSC transition to differentiated cell types including: Transforming Growth Factor Beta-2 (TGF- β 2), Sry-Related High Mobility Group Box Containing Gene 9 (SOX9), Paired Box Gene 9 (Pax9) and Runt-Related Transcription Factor 2 (Runx2). *In vivo* exposure of medaka embryos to TCDD results in a dose-dependent decrease in transcription factor expression for all genes examined at 10 day post fertilization (dpf) using both RT-PCR and/or *in situ* hybridization. A marked reduction in Col2a1 protein expression, a marker of chondrocyte differentiation, is additionally observed at 10 dpf in medaka hypural cartilage consistent with an observed reduction in hypural cartilage deposition.

PL 79 METHYL MERCURY-INDUCED PERTURBATION OF NEURAL DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS OVER TIME DESCRIBED BY TRANSCRIPTOMICS.

P. T. Theunissen[1, 2], J. L. Pennings[1], J. C. Kleinjans[2], J. F. Robinson[1] and A. H. Piersma[1, 3]. [1]*Laboratory for Health Protection Research, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands,* [2]*Department of Health Risk Analysis and Toxicology, University of Maastricht, Maastricht, Netherlands and* [3]*Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands.*

Current chemical hazard assessment for developmental toxicity requires high numbers of experimental animals. Alternative developmental toxicity assays are highly desirable to reduce animal use. For screening effects on neural development, we previously developed a dynamic in vitro model which makes use of neural cell differentiation of pluripotent murine embryonic stem cells (Reprod. Toxicol. 2010 29:383-92). To further characterize this neural differentiation model and to improve detection of developmental toxicants, gene expression patterns within the model were studied. A transcriptomics study was performed to study whole genome expression changes during the first 7 days of the protocol. Statistical analysis was performed using R. Pathway enrichment analysis of GO terms was performed using DAVID and T-profiler. Analysis of gene expression using PCA showed a time dependent track in unexposed controls, describing the process of neural differentiation. This was confirmed for specific gene sets using pathway enrichment analysis. They revealed downregulation of blastocyst and trophectoderm related genes and upregulation of neural development related gene sets over time. Furthermore, the developmental toxic effects of methyl mercury (MeHg) on neural differentiation over time were assessed. MeHg was shown to induce deviation from the predefined differentiation track. The compound inhibited proliferation related pathways and induced neural related pathways over time. This system appears promising for studying compound effects on neural differentiation in a mechanistic approach.

PS 80 VALIDATION OF THE *CANDIDA ALBICANS* DELAYED-TYPE HYPERSENSITIVITY (DTH) MODEL IN THE B6C3F1 MOUSE FOLLOWING EXPOSURE TO AZATHIOPRINE (AZA), CYCLOPHOSPHAMIDE (CPS), CYCLOSPORIN A (CSA), DEXAMETHASONE (DEX), OR BENZO[E]PYRENE (B[E]P) FOR 28 DAYS.

M. J. Smith, C. E. McLoughlin, W. Auttachoat and K. L. White. *Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA.*

Although numerous models are in use to evaluate the immunotoxic effects of a xenobiotic on cell-mediated immunity (CMI), no single holistic model for evaluating such effects on the DTH response has gained widespread acceptance. The *C. albicans* DTH model has been suggested recently as a more appropriate model for evaluating effects on the DTH response in mice than models using either sheep erythrocytes (sRBC) or keyhole limpet hemocyanin (KLH) as sensitizing antigens. The purpose of these studies was to validate the *C. albicans* DTH model for its ability to detect suppression (or the lack thereof) of CMI following exposure for 28 days to well-characterized immunosuppressive drugs having different mechanisms of action. The compounds evaluated included AZA, CPS, CSA, DEX, and the non-immunotoxic compound B[e]P. Results from initial studies demonstrated that the *C. albicans* DTH model was able to detect suppression of CMI for each known immunotoxicant, with statistically significant decreases in the DTH response observed following exposure to AZA (at 30 mg/kg but not at 10 mg/kg), CPS (at 30 mg/kg but not at 10 mg/kg), CSA (at 1 mg/kg, the lowest dose evaluated), or DEX (at 0.3 mg/kg but not 0.1 mg/kg). As expected, B[e]P exposure for 28 days at doses up to 40 mg/kg did not suppress the DTH response. These results indicate that the *C. albicans* DTH assay in the B6C3F1 mouse was capable of appropriately classifying each test article as to its immunotoxic effects on CMI. Furthermore, these results further validate the use of this model in immunotoxicity testing. Supported in part by NIEHS Contract ES 05454.

PS 81 A RETROSPECTIVE OF THE REGULATORY USE OF THE LOCAL LYMPH NODE ASSAY FOR THE NOTIFICATION OF NEW CHEMICALS IN EUROPE.

S. Casati, A. Angers-Loustau and L. Tosti. *European Commission (Joint Research Centre), Ispra, Italy.* Sponsor: D. Basketter.

We have monitored the regulatory use of the LLNA for chemicals registration from the time of its adoption as stand alone method at the OECD to 2008. For this, we screened the New Chemicals Database (NCD) which was managed during this period by the former European Chemicals Bureau (ECB) at the European Commission Joint Research Centre (JRC). The NCD comprises chemicals notified after 1981, where registered data have been derived according to regulatory standards, including GLP and predominantly according to official test methods. The database was searched to extract records for which the information for skin sensitisation labelling was based on results derived with the LLNA. The details of these records were extracted and pooled, for a total of 545 entries, and evaluated with regards to the extent of use of the LLNA over time and countries, as well as analysing the information derived on critical aspects of the procedure e.g strain and amount of animals used, lymph node processing, solvent and doses selected, stimulation indices, and assessing their level of compliance to the OECD 429. Additional information on labelling was extracted, and its significance for the potential of the widespread use of the reduced version of the LLNA is discussed on the poster.
Disclaimer: The views expressed in this poster are purely those of the authors, and should not be regarded as an official position of the European Commission.

PS 82 COMPARISON OF 3H-THYMIDINE INCORPORATION AND NON-RADIOACTIVE CELL COUNTEND AS ENDPOINTS OF THE LOCAL LYMPH NODE ASSAY (LLNA) IN ROUTINE TESTING.

S. N. Kolle, A. O. Gamer, A. Schrage, N. Honarvar, V. Strauss, R. Landsiedel and B. van Ravenzwaay. *Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany.* Sponsor: A. Van Cott.

The local lymph node assay as described in the OECD guideline 429 is based on measuring lymph node cell proliferation by 3H-Thymidine incorporation into the lymph node. Alternative endpoints were evaluated recently (Basketter et al. 2008). The evaluation of cell proliferation by the cell counts in the single cell suspensions produced from the ear lymph nodes (LLNC) as described by Vohr et al. (2000) proved to be useful for evaluation of LLNA if the cut off stimulation index (SI) for positive tests was adjusted to 1.5, reflecting the overall range of cell count increase (Ehling et al. 2005). This has been demonstrated in a multi-center study and a study on 13 epoxy resin constituents (epoxides and amines) (Gamer et al. 2008). We present data from routine studies with a 170 industrial chemicals and agrochemical formulations (54% were identified as sensitizers, 74% as irritants, and 46% as both sensitizers and irritants) and additionally the 24 substnces of the performance standard list obtained during routine testing using both the LLNC and the radioactive endpoints (dpm). Results indicate that there is a correlation between LNCC and dpm values. Furthermore equivalent estimated concentrations (ECs) for the prediction of skin sensitizing potency are obtained in the majority of cases with both measurements. The results indicate that if an adjusted reference SI is used for LNCC, equivalent designation of test substances as sensitizers were obtained compared with 3H-thymidine incorporation. Overall, 3H-thymidine incorporation identified more substances as weak and LNCC identified more as moderate or strong.

PS 83 VEHICLE-DEPENDENT EFFECTS ON HEXYLCINNAMALDEHYDE RESPONSES IN THE LLNA.

G. L. DeGeorge, M. Carathers, J. Tao and D. R. Cerven. *MB Research Laboratories, Spinnerstown, PA.*

Hexylcinnamaldehyde (HCA) is the default, preferred positive control substance in the Local Lymph Node Assay (LLNA), due to its moderately potent dermal sensitizing properties. We have tested the effects of use of varying vehicle solvents for HCA on the endpoints of Stimulation Index (SI), lymphotype subsets (immunophenotyping; IP), and irritation as measured by ear swelling. Herein, we show that the choice of vehicle does biologically and statistically significantly (P>0.05) impacts the HCA endpoint values, most importantly, the SI. HCA at 25% was evaluated in all vehicles (AOO, DMSO, Acetone, Ethanol and DAE433, petrolatum, PG, etc.), and compared to naïve or untreated controls, as appropriate. Most importantly, some vehicles (besides the default AOO) that are commonly used were more prone to causing or enhancing irritation induced by vehicle-alone treatment or either decreased or increased SI values and variability when compared to AOO. Generally, DAE433 was a 'better' vehicle than DMSO alone, resulting in lower background proliferation and less irritation (ear swelling day1 through Day 6). Petrolatum gave good results as a vehicle for 25% HCA with an SI=10.3, comparable to AOO SI=9.8 and DMF SI=9.9. Acetone and PEG had significantly higher SI values and B:T cell ratios than DMSO and AOO. DMSO and DMA had the lowest SI values for 25%HCA, at 7.8 and 5.8, respectively. In addition, DMSO was significantly irritating to the ears of mice as a vehicle, and caused very pronounced ear swelling when used as a vehicle for HCA (>15% increase). In conclusion, the vehicle chosen for the LLNA can significantly affect multiple endpoints of interest in the assay, especially the Stimulation Index, and that this variability should be taken into account when testing similiar or borderline test substances in vehicles other than AOO.

PS 84 RESPIRATORY TRACT RESPONSES IN WISTAR AND BN RATS, SENSITIZED AND CHALLENGED BY INHALATION WITH THE CONTACT ALLERGEN DINITROCHLOROBENZENE (DNCB).

J. van Triel[1], J. Arts[1, 2] and F. Kuper[1]. [1] *Toxicology and Applied Pharmacology, TNO, Zeist, Netherlands and* [2] *Technology & Engineering, Akzo Nobel, Arnhem, Netherlands.* Sponsor: R. Woutersen.

All chemical respiratory allergens studied sofar can also induce skin sensitization/allergy in test animals. The question is if in turn contact (skin) allergens can induce allergy in the respiratory tract. The contact allergen dinitrochlorobenzene (DNCB) was tested first in Th2-prone Brown Norway (BN) rats, using a protocol that successfully identified chemical respiratory allergens like trimellitic anhydride. Dermal sensitization induced DNCB-specific IgG in serum. A subsequent single inhalation challenge with DNCB did not provoke apnoeic breathing or allergic inflammation in the respiratory tissues (signs of respiratory allergy), but the allergy-associated genes for Ccl2 (MCP-1), Ccl4 (MIP-1beta), Ccl7 and Ccl17 were upregulated in lung tissue. Next, DNCB was tested in Th1-prone Wistar rats. Again, a single inhalation challenge in sensitized rats did not provoke apnoeic breathing, but induced a minimal lymphocytic infiltrate in the nasal tissues and larynx. Repeated inhalation challenges (twice a week for 4 weeks) in Wistar rats induced DNCB-specific IgG antibodies in serum and a pronounced, predominantly lymphocytic, inflammation in the nasal tissues and larynx. The inflammation may be the upper respiratory tract analogue of hypersensitivity pneumonitis/allergic alveolitis. The relevance of these findings to man, and possible progression of the airway inflammation should be investigated to support or dismiss discrimination between contact and respiratory allergens in relation to respiratory allergy.

PS 85 STEPS TOWARDS THE DEVELOPMENT OF AN INTEGRATED APPROACH FOR THE PREDICTION OF SKIN SENSITIZATION POTENTIAL USING DATA FROM SEVERAL ALTERNATIVE TEST SYSTEMS.

C. Ryan[1], J. Jaworska[2], P. Kern[2], J. Chaney[1] and F. Gerberick[1]. [1] *Procter & Gamble Company, Cincinnati, OH and* [2] *Procter & Gamble Company, Brussels, Belgium.*

A number of different in vitro, in chemico and in silico methods have been developed to examine the skin sensitization potential of chemicals. However, it is the general view that a single test method cannot replace the current animal tests for such a complex endpoint. Therefore, development of an integrated approach is needed which incorporates data from several test systems for the prediction of the skin sensitization effects of chemicals. Two approaches are being taken to develop a prediction model that would quantify in some way data for epidermal bioavailability generated in silico, peptide reactivity from an in chemico assay and dendritic cell activation from an in vitro U937 cell line-based test system. The first approach uses recursive partitioning methodology in order to build a classification tree. The algorithm starts with the complete data set and seeks to partition it into separate subgroups of more homogeneous subsamples. An optimal split variable and a corresponding threshold value are selected. Then the sample is split into two subgroups: (1) observations with split variable values less than the threshold, and (2) observations with split variable values greater than the threshold. This same binary partitioning is then applied separately to the two subsets. The process is repeated until the increase in subset homogeneity and/or the resulting sample sizes are too small to continue. The second approach explores use of a Bayesian network which in addition to integrating data, can serve as a decision tool for guiding testing strategies based on identification of the most informative tests. Both approaches hold promise as a step forward towards making efficient use of alternative data.

PS 86 DIFFERENTIATION OF PROHAPTENS FROM DIRECT ACTING CONTACT CHEMICAL ALLERGENS USING A CYTOCHROME P450 REDUCTASE DEFICIENT MOUSE MODEL.

I. Chipinda, F. M. Blachere, S. E. Anderson and P. D. Siegel. *HELD, CDC/NIOSH, Morgantown, WV.*

The murine local lymph node assay (LLNA) is a well accepted, validated method for identification of chemical contact allergens. Both direct acting haptens and prohaptens (requiring metabolic activation) can be identified, but not differentiated by this assay. The objective of this study was to assess the utility of a pan microsomal metabolic deficient mouse (CPR low/low) to distinguish between direct acting haptens and prohaptens in the LLNA. LLNA cell proliferation was compared in C57BL/6J (B6) wild type vs. homozygous CPR low/low mouse, congenic with the B6 strain, having a hypomorphic NADPH-cytochrome P450 reductase (CPR) gene resulting in low microsomal enzyme activity. The known prohaptens, benzo(a)pyrene (BaP), carvone oxime (CVO) and paracetamol (PCM) and direct binding haptens, oxazolone (OX), 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (EtOX), and N-acetylbenzoquinoneimine (NABQI) employed in the present study. Skin microsomes from the wild type (WT) and CPR low/low homozygous (HM) and heterozygous (HT) knock-out (KO) mice were assayed and compared for CPR activity. Lymphocyte proliferative responses to BaP, CVO and PCM were significantly abrogated by 36.4%, 45.2% and 50.8%, respectively; in CPR low/low KO mice versus WT mice; while the lymphocyte proliferative responses to the direct

acting haptens OX, EtOX and NABQI were comparable. CPR activity, determined as Units/mg protein was determined to be significantly lower in the CPR low/low KO mice compared to the WT. Results of the present study suggest potential utility employing the LLNA in the CRP low/low mouse in conjunction with WT to differentiate pro- vs. direct acting haptens.

PS 87 PLASMACYTOID DENDRITIC CELL-BASED ASSAY AS AN *IN VITRO* ALTERNATIVE FOR CHEMICAL ALLERGENICITY SCREENING.

M. Spratt, M. Klausner, S. Ayehunie and M. Child. *MatTek Corp, Ashland, MA.* Sponsor: P. J.

Human dendritic cells (DC) have been used as an alternative to existing animal models utilized throughout industry to monitor products for contact sensitization. Such methods are necessary to comply with the ban on animal testing imposed by the Cosmetics Directive in the EU. In this study, we investigated whether CD86 expression in plasmacytoid DC (pDC) can be used to identify contact allergens. Human DC were generated from CD34+ progenitor cells and the pDC fraction (CD123+/CD11c-) was harvested using FACS sorting. The pDC were exposed to an expanded list of chemical allergens (n=49) or irritants (n=42). Concentrations of each chemical that resulted in >50% viability as determined by FACS analysis of propidium iodide stained cells were used. Allergens were identified based on stimulation index (SI) calculated by the fold increase in CD86 expression levels. A material that had an SI ≥1.5 in at least 50% of the pDC donors (n=2-5 donors) was considered an allergen. For 71 of the 91 materials tested, historical mouse local lymph node assay (LLNA) and human clinical data were available. Using the in vitro pDC assay, CD86 expression increased ≥1.5 fold for 37 of 39 allergens but not for 26 of 32 non-allergens. Based on these results, a prediction model was developed to classify chemicals as allergens or non-allergens. The in vitro assay performance was sensitivity=95%, specificity=81%, and accuracy=89%; these results were slightly better than those obtained using the LLNA assay: sensitivity=85%, specificity=84%, and accuracy=85%. Transferability of the test method was evaluated using 7 test articles in 3 laboratories. The results showed that all samples were correctly identified in the 3 labs. In conclusion, the CD86 expression level in pDC appears to be a sensitive and specific predictor of allergenicity. The pDC assay is advantageous because high throughput screening of chemicals is possible, donor-to-donor variation can be monitored, the cells are of human origin, and the assay is cost effective.

PS 88 CELL LINE-BASED PREDICTION OF SENSITIZATION BY COUPLING KERATINOCYTE METABOLISM AND DENDRITIC CELL ACTIVATION.

J. Hennen[1], P. Aeby[2], C. Goebel[3], T. Schettgen[4], A. Oberli[5], M. Kalmes[1] and B. Blömeke[1]. [1]*Environmental Toxicology, University Trier, Trier, Germany,* [2]*Consultant, Marly, Switzerland,* [3]*Procter & Gamble Co., Darmstadt, Germany,* [4]*RWTH Aachen University, Aachen, Germany and* [5]*Federal Office of Public Health (FOPH), Bern, Switzerland.*

Frequency of allergic contact dermatitis is constantly increasing and extended knowledge about the sensitizing process is essential for the development of preventive strategies. Many compounds are not immunogenic themselves but are activated outside or inside the skin by non-enzymatic oxidation (prehaptens) or enzymatic transformation (prohaptens, e.g. cytochrome P450) prior induction of an immune response. This necessary bioactivation step has not yet been actively integrated in a cell line-based prediction approach. We cocultured HaCaT keratinocytes with THP-1 as dendritic cell-like cells (ratio 1:0.75). The sensitizing potential can be determined by analyzing the maturation markers CD86 and CD54 on cocultured THP-1 cells. Coculturing augmented cytochrome P450 1 activity in HaCaT cells after treatment with chemicals. The assay allowed the differentiation between the irritant sodium dodecyl sulfate and allergens without influencing cell viability. We achieved the sensitivity to testing prohaptens and their differentiation from prehaptens and haptens using lipopolysaccharide, 2,4-dinitrochlorobenzene, eugenol and isoeugenol. For the prohapten eugenol the upregulation of the CD86 expression was 4-fold higher in the presence of HaCaT. In concordance with the hapten concept, responses to 2,4-dinitrochlorobenzene and to the prehapten isoeugenol were not modified or even slightly reduced under these terms. A coculture assay with HaCaT and THP-1 cells is easy to perform, reproducible, avoids donor variance, and allows the detection of prohaptens under rather physiological conditions. Thus, this assay is a reasonable approach to search for the sensitizing potential of compounds, and as one part of an integrated testing strategy it may replace the local lymph node assay.

PS 89 DEVELOPMENT OF A MOUSE MODEL TO ASSESS THE ALLERGENICITY OF HYDROLYSED COW'S MILK BASED INFANT FORMULAE.

B. C. van Esch[1], J. H. Van Bilsen[2], P. V. Jeurink[3], J. Garssen[3], J. Smit[4], R. Pieters[4] and L. M. Knippels[3]. [1]*Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands,* [2]*Quality of Life, TNO, Zeist, Netherlands,* [3]*Immunology, Danone Research Centre for Specialized Nutrition, Wageningen, Netherlands and* [4]*IRAS, Utrecht University, Utrecht, Netherlands.*

The EC-directive 2006/141/E requires objective and scientifically verified data to claim hypoallergenicity of hydrolysed formulae. However, no validated animal models are currently available to assess the potential residual sensitizing capacity, although guinea pig assays are frequently used. This study aims to validate a recently developed mouse model for cow's milk allergy to assess the potential allergenicity of hydrolysed cow's milk-based infant formulae. To that end, a multicenter transferability study was performed to introduce the model in 4 independent research centers. C3H/HeOuJ mice (3-4 weeks, Charles River) were sensitized by oral administration of whey (2 and 20 mg) at weekly intervals for 5 weeks. One week after the last sensitization the acute allergic skin response (ear swelling at 1 hr) and anaphylactic symptoms were determined upon intradermal ear injection of whey. Subsequently, mice were challenged orally with 50 mg whey and blood samples were taken after 30 minutes. Serum was analyzed for whey-specific immunoglobulins and mMCP-1. All protocols, test substances, and procedures were standardized. All participating research laboratories detected elevated levels of whey-specific IgE/IgG1/IgG2a and serum mMCP-1 as a reflection of mast cell degranulation. Acute allergic skin responses were observed in 3 out of 4 research centers and anaphylactic symptoms were present at all 4 research centers. These results are indicative of good interlaboratory transferability. In the next phase of the validation process, whey hydrolysates will be included to assess whether the proposed mouse oral sensitization model is suitable for evaluation of hypoallergenic cow's milk formulae.

PS 90 PREFERENTIAL REACTIVITY OF CONTACT AND RESPIRATORY LOW MOLECULAR WEIGHT CHEMICAL ALLERGENS UNDER COMPETITIVE CONDITIONS.

J. F. Lalko[1, 3], I. Kimber[1], R. J. Dearman[1], F. Gerberick[2], A. Api[3] and L. W. Smith[3]. [1]*The University of Manchester, Manchester, United Kingdom,* [2]*Procter & Gamble Company, Cincinnati, OH and* [3]*RIFM, Inc., Woodcliff Lake, NJ.*

Low molecular weight chemicals are capable of causing allergic diseases of the skin and respiratory tract. Individual materials are typically, though not always, associated with one form of disease or the other – generating a Th1 or Th2 type response. The reason for this divergence is unclear; however chemical interaction with proteins is an important common step. Using a peptide reactivity model, the reactivity of reference skin (dinitrochlorobenzene [DNCB], dinitrofluorobenzene [DNFB]) and respiratory allergens (toluene diisocyanate [TDI], methylene diphenyl diisocyanate [MDI]) was investigated. Of particular interest was determining if there exist preferences for binding to either cysteine (Cys) or lysine (Lys). One set of assay was conducted by reacting synthetic peptides containing either Cys or Lys alone with an excess of test chemical. In order to evaluate the effect of competition, assays were conducted by preparing reaction mixtures of these same peptides in various concentrations relative to the other. The ratios utilized were 1:1, 3:1, 6:1 and 9:1; in each case the total peptide content was constant. The samples were analyzed for depletion of peptide by HPLC/UV after 24 h incubations. When incubated with single peptides, DNCB and DNFB were observed to have increased reactivity to Cys when compared to both TDI and MDI. Although DNCB lacked significant reactivity, DNFB and the isocyanates had comparable reactivity with the Lys peptide. Under competitive conditions, however, both DNCB and DNFB (contact allergens) showed preferential binding to Cys; while TDI and MDI (respiratory allergens) exhibited preferential binding to Lys. The preferences were most evident at the 6:1 and 9:1 reaction ratios; pairwise comparisons showed that this was not due to concentration dependence. The observed differences may be relevant for the ability of these chemicals to induce divergent allergic responses.

PS 91 ALLERGEN EXPOSURE RESULTS IN CHANGES IN THE LEVEL OF EXPRESSION AND FREQUENCY OF B220+ LYMPHOCYTES.

S. Khan[1], I. Kimber[1], R. J. Dearman[1], J. F. Lalko[1, 2] and A. Api[2]. [1]*The University of Manchester, Manchester, United Kingdom and* [2]*RIFM Inc., Woodcliff Lake, NJ.*

The murine local lymph node assay (LLNA) is a validated alternative for the predictive identification of skin sensitizing chemicals. As with other predictive test methods, false-positives are known to occur, particularly to a small minority of skin

irritants. While in practice these typically do not present interpretive difficulties, there is interest in continuing to improve the performance of the LLNA by eliminating or reducing false-positives. One approach, which has met with some success, is to align the LLNA with monitoring the frequency of B220$^+$ lymphocytes in skin draining lymph nodes (LN). In the current experiments (n=3), CBA/Ca mice have been treated topically with the contact allergen dinitrochlorobenzene (DNCB; 0.25%), the skin irritant benzalkonium chloride (BZC; 2%) or with vehicle (acetone) alone using the standard LLNA dosing regimen. Draining LN have been isolated and the level of expression and the frequency of B220$^+$ cells measured by flow cytometry. Treatment with both chemicals resulted in a marked increase in total cellularity (increasing from 4.1±0.6 to 14.0±5.1 or 27±7.2 x10^6 cells/LN for BZC and DNCB, respectively). Baseline (vehicle) B220 cell frequencies were relatively stable (mean 6%; range 4.6-6.9%) whereas expression levels (mean fluorescence intensity, MFI) ranged from 105 to 573 (mean 304). Only DNCB provoked marked increases in both the level of B220 expression and the frequency of B220$^+$ cells. Thus, a 2.6 fold increase in B220 expression and a 3.3 fold increase in B220$^+$ cell numbers were recorded on average following exposure to DNCB. Identical treatment with BZC resulted in little change in these parameters (<1.5 fold differences). These data demonstrate that contact allergens, but not skin irritants, stimulate changes in B220$^+$ cells in the draining LN. As increases in both the frequency and the level of expression of the marker are recorded, such changes are likely to be due in part to activation of resident B220 cells rather than the influx of circulating B220 cells.

PS 92 THE SURVIVAL OF DENDRITIC CELLS IS CONTROLLED BY THE TRANSCRIPTIONAL FACTOR NRF2 IN RESPONSE TO CHEMICAL SENSITIZERS.

S. Kerdine-Römer, Z. El ALi, M. Damiens and M. Pallardy. *Toxicologie, Université Paris Sud-INSERM UMR 996, Châtenay-Malabry, France.*

Dendritic cells (DC) are professional antigen presenting cells playing a major role in the induction of primary immune response. After the exposure of DC to skin contact sensitizers, such as nickel (NiSO4) or dinitrochlorobenzene (DNCB) an up-regulation of phenotypic markers (CD86, CD83, HLA-DR and CD40), cytokine secretion (IL-6, IL-12p40, IL-8) and activation of the signaling pathways such as Mitogen Activated Protein Kinases are observed. These molecules generate a chemical stress that can be perceived as a danger signal by DC, leading to their maturation. Among signaling pathways known to be redox-sensitive, the Nrf2/Keap1 pathway is central for the detection of electrophilic molecules. In the absence of a chemical stress, Keap1 associates with Nrf2 and targets it to degradation. In the presence of an electrophilic molecule, Keap1's conformation is modified leading to Nrf2 translocation to the nucleus and transcription of its target genes [heme-oxygenase 1, NADPH quinone oxydoreductase, glutathione-s-transferase]. To study the role of the transcriptional factor Nrf2 in response to chemical sensitizers, DC derived from monocytes (Mo-DC) were treated with NiSO4, DNCB or cinnamaldehyde (CinA) for different times. In response to these three chemical sensitizers, Nrf2 expression was measured by Western blot. Accumulation of Nrf2 protein was observed after 4 hours of stimulation. To evaluate the role of Nrf2 in cell survival, Mo-DC were transfected with small interfering RNA (siRNA) to invalidate in vitro nrf2 transcripts and were then treated with NiSO4 or DNCB or CinA for 18h. Results showed an increase of apoptotic and secondary necrotic cells, measured by Annexin V (AnV) and 7-AAD staining. The results showed also an increase of secondary necrosis (AnV+/7-AAD+) in dendritic cells invalidated for nrf2. These results suggest that the transcriptional factor Nrf2 plays a role in cell survival in response to chemical sensitizers.

PS 93 CHRONIC EXPOSURE OF MICE TO CHEMICAL ALLERGEN IS ASSOCIATED WITH A TH17 PHENOTYPE.

R. J. Dearman, M. D. Hayes and I. Kimber. *Faculty of Life Sciences, Manchester University, Manchester, Greater Manchester, United Kingdom.*

The development of immune and allergic responses is orchestrated by T helper (Th) cell subsets. For some years, it has been recognised that Th1 and Th2 subsets mediate cellular and humoral immunity, respectively, and it has been shown these subsets play important roles in the development of chemical allergy. Thus, prolonged (13 day) exposure of BALB/c strain mice to the contact allergen 2,4-dinitrochlorobenzene (DNCB) results in the preferential activation of Th1 cells whereas the respiratory sensitiser, trimellitic anhydride (TMA), selectively stimulates Th2 cells. More recently, a new subset, Th17 cells, has been implicated in the pathogenesis of autoimmune and inflammatory diseases. Th17 cells express several different isoforms of IL-17 (IL-17A and F homodimers and IL-17A/F heterodimers) and IL-22 (which targets non-immune cells). BALB/c strain mice were exposed topically to 1% DNCB, 10% TMA or to vehicle (acetone:olive oil; 4:1) alone. Six to 72h after a single exposure, or 13 days after repeated exposure, draining (auricular) lymph nodes were extracted and pooled on an experimental group basis (n=3-5). Single cell suspensions were prepared and cultured for up to 120 h and supernatants analysed by cytokine-specific enzyme-linked immunosorbant assay (ELISA). A single topical exposure to the allergens caused a transient up-regulation of the secretion of IL-17A, IL-17F and, to a lesser extent, IL-17A/F; reaching maximal expression at 6h and 48h post exposure in response to DNCB and TMA respectively. However, repeated exposure only to DNCB resulted in marked increases in production of the heterodimer IL-17A/F and of IL-22. The preferential expression of the heterodimer following chronic exposure to DNCB is indicative of the induction of a polarised Th17 cell population which may play a role in the immunopathology of the disease. The early, transient production of IL-17 induced by both allergens is more likely to be a result of activation of "sentinel" cells of the innate immune system such as various types of natural killer (NK) cells.

PS 94 ALLERGEN-INDUCED LANGERHANS' CELL AND DENDRITIC CELL MIGRATION: COMPARISONS BETWEEN 2, 4-DINITROCHLOROBENZENE AND 2, 4-DINITROTHIOCYANOBENZENE.

M. Cumberbatch, C. F. Portsmouth, I. Kimber and R. J. Dearman. *University of Manchester, Manchester, United Kingdom.*

There is some debate as to the sensitising potential of 2,4-dinitrothiocyanobenzene (DNTB), a chemical that has been reported to exhibit tolerogenic properties. We have shown previously that the structurally related chemicals 2,4-dinitrochlorobenzene (DNCB) and DNTB provoke equivalent contact hypersensitivity responses in BALB/c strain mice, measured as a function of challenge induced increases in ear thickness. Interestingly, whereas DNCB stimulates epidermal Langerhans' cell (LC) migration within 4h of exposure, DNTB fails to induce LC mobilisation, even after 24h. However, both chemicals induce the accumulation of similar numbers of dendritic cells (DC) in the draining lymph node (DLN), as assessed morphologically. Furthermore, DNCB-induced DC accumulation requires both tumour necrosis factor-α and interleukin (IL)-1β, whereas the response provoked by DNTB requires only IL-1β. We have exposed BALB/c mice topically to 1% DNTB or 1% DNCB and monitored the arrival in DLN 18h later of different cutaneous DC subsets using flow cytometry. DNTB and DNCB caused the accumulation of equivalent numbers of CD11c+/MHC class II+ DC. Furthermore, the percentage of cells that were langerin (CD207)+ or CD11c+ remained unaltered by chemical treatment, but DLN cellularity increased significantly. Thus, both chemicals were associated with similar increased numbers of CD207+ or CD11c+ DC/node. All CD207+ DC were CD11c+/MHC class IIhigh and no differences were observed between DNTB and DNCB with respect to numbers of CD207-/CD11c+ DC or MHC class II+ cells. These data are consistent with current views on the contribution of cutaneous DC subsets to contact sensitisation: that the identification of LC and dermal DC subsets in DLN is complex, and that at 18h following exposure to chemical allergen the arrival of LC in LN may be preceded by the more rapid influx of dermal DC.

PS 95 DIFFERENTIAL REGULATION OF LANGERHANS' CELL (LC) MIGRATION BY THIOREDOXIN AND LACTOFERRIN.

R. Almond[1], M. Cumberbatch[1], G. del Val[2], I. Kimber[1] and R. J. Dearman[1]. [1]*University of Manchester, Manchester, United Kingdom and* [2]*Syngenta Biotech, Bracknell, United Kingdom.*

Epidermal Langerhans' cells (LC) play a critical role in the regulation of skin immune and allergic responses. LC migration, including that stimulated by allergen, is dependent upon cytokine signals, provided by tumour necrosis factor-α (TNF-α) and interleukin (IL)-1β. We have now examined the ability of two human recombinant proteins with reported immunomodulatory properties to influence LC migration : thioredoxin (Trx), a nonglycosylated (12kDa) redox-active protein produced in E. coli, and lactoferrin (LF), a glycosylated (79.5kDa) iron-binding protein raised in rice. LC migration in response to chemical contact allergen (oxazolone; Ox, 0.5%) or to intradermal injection of recombinant murine TNF-α or IL-1β (50ng) was assessed in BALB/c mice. Mice were pretreated by topical application of 0.5μg of TRX or 12μg of LF formulated in aqueous cream per site 2 h prior to treatment with the migratory stimuli. LC densities were assessed in epidermal sheets by immunofluorescent staining for major histocompatibility complex (MHC) class II or Langerin. Topical application of Trx inhibited significantly Ox- and TNF-α-induced LC migration, but failed to impact on IL-1β-induced migration. Use of mutant forms of Trx demonstrated that this ability was redox-independent. In contrast, pre-treatment with LF prevented LC mobilisation stimulated by Ox and IL-1β treatment, but was without significant effect on migration provoked by TNF-α. Inhibition of LC migration by TRX was selective for IL-1β-dependent stimuli (contact allergen and TNF-α) whereas TNF-α-dependent migration (that provoked by contact allergen and IL-1β) were inhibited by LF. These data

suggest that TRX possesses novel immunomodulatory properties as a result of its ability to modulate IL-1β production and/or signalling. In contrast, LF inhibits the activity of TNF-α. The bioactivity of these molecules following simple topical application demonstrates that these proteins can access the viable epidermis and may represent an opportunity for the development of novel anti-inflammatory reagents.

PS 96 PROTEIN ALLERGENICITY AND DIGESTIBILITY: COMPARISONS OF PEPSIN AND CATHEPSIN.

E. Foster, I. Kimber and R. J. Dearman. *University of Manchester, Manchester, United Kingdom.*

An association between protein allergenicity and resistance to pepsin digestion has been reported previously, however, such is not complete with examples of labile allergens and resistant non-allergens observed. Given the central role of antigen presenting cells, such as dendritic cells (DC), in the development of immune and allergic responses, the stability of allergens to intracellular processing may be more relevant than resistance to pepsin digestion. We have characterised the expression by DC of cathepsins (proteolytic enzymes), and compared the proteolytic activity of the most highly expressed cathepsin with pepsin. Cathepsin expression in bone marrow-derived DC (BMDC) and in mesenteric lymph node DC derived from BALB/c strain mice was characterised by flow cytometry. BMDC expressed detectable levels of cathepsin D, E and S, with cathepsin D being the most highly expressed, with a similar pattern observed in DC isolated from mesenteric lymph nodes. Digestion studies revealed that the allergens β-lactoglobulin (BLG), hen egg lysozme (HEL) and ovalbumin (OVA) were relatively resistant to pepsin, although bovine serum albumin (BSA) was labile, as were the non-allergens hemoglobin (HB) and horseradish peroxidise (HRP). In contrast, all 4 allergens were stable to overnight digestion with cathepsin D, although fragments were observed for BSA. HB was labile to cathepsin D whereas fragments were recorded for HRP. As intracellular digestion occurs under reducing conditions, the impact of prior chemical reduction was explored. Reduced BLG and OVA were labile to pepsin and fragments were observed following cathepsin D digestion. Generally, allergens were more stable than non-allergens to digestion by both enzymes and chemical reduction increased lability. Although a wider range of allergens and non-allergens needs to be examined, these data suggest that allergenicity correlates more strongly with resistance to digestion by cathepsin D than with pepsin.

PS 97 INHALATION OF *ORTHO-PHTHALALDEHYDE* VAPOR CAUSES SYSTEMIC SENSITIZATION AND ALLERGIC INFLAMMATION IN THE LYMPH NODES, NASAL MUCOSA, AND LUNG OF MICE.

V. J. Johnson, W. Wang, K. Fluharty, B. Yucesoy and J. S. Reynolds. *CDC/NIOSH, Morgantown, WV.*

Ortho-Phthalaldehyde(OPA) is increasingly being substituted for glutaraldehyde as a high-level disinfectant for sensitive medical devices. However, toxicological data on OPA safety is lacking. Safety concerns for glutaraldehyde include acute nasal toxicity and sensory irritation as well as sensitization leading to occupational rhinitis and asthma. Since OPA is a dialdehyde like glutaraldehyde and functions as a disinfectant due to its high reactivity for biological macromolecules, it is reasonable to expect similar respiratory hazards. Several case reports have been published supporting this hypothesis showing development of respiratory hypersensitivity reactions in healthcare workers and patients exposed to OPA. The purpose of this study was to determine if OPA is a respiratory sensitizer using a murine model. Mice were exposed via inhalation to OPA vapor 4 hrs/day for 3 days; rested for 11 days; challenged with OPA vapor 4 hrs/day for 3 days and then sacrificed 24 hrs after the final exposure. Lungs, nasal mucosa, head-draining lymph nodes (LN) and serum were collected and processed for cytokine gene expression and flow cytometry. OPA-specific antibodies were detected in the serum of exposed mice. OPA inhalation induced a dose dependent increase in LN IL-4 expression and B-cell proliferation. Importantly, there was a concomitant increase in IgE+ B-cells. Strong Th2 cytokine expression in the nasal mucosa increased with OPA dose whereas the inverse was observed for IFNγ expression, a cytokine expression pattern commonly observed in response to respiratory allergens. Th2 cytokine expression was also increased in the lung although to a lesser extent. These data demonstrate that OPA inhalation induces a predominant Th2 cytokine response in the respiratory tract and draining LN. Importantly, OPA inhalation induced isotype switching to IgE in the draining LN supporting the development of an allergic immune response. Overall, this study suggests that inhalation of OPA vapor in mice can induce respiratory allergy.

PS 98 CONTACT SENSITIZING POTENTIAL OF HEPTACHLOR IN FEMALE BALB/C MICE.

R. P. Frawley[1], W. Auttachoat[2], M. J. Smith[2], R. D. Brown[2], T. L. Guo[2], K. L. White Jr[2] and D. R. Germolec[1]. [1]*National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC and* [2]*Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA.*

Heptachlor (HPT) is a chlorinated cyclodiene insecticide that was used extensively in the 1950's through 1980's in agriculture, and in commercial and domestic buildings. Banned by the Environmental Protection Agency (EPA) for most uses (except for fire ant control in underground power cable boxes) since 1988, low levels of HPT residues persist in the environment. Human exposure can occur through contact with contaminated soil or building materials; consumption of contaminated meat, fish or dairy products; or production and application of HPT insecticides. The objective of this study was to evaluate the sensitization potential of HPT in female BALB/c mice following dermal exposure using the ICCVAM-validated local lymph node assay (LLNA) in combination with a measurement of irritancy, and the mouse ear swelling test (MEST). Sensitization was evaluated at concentrations that did not induce overt toxicity. HPT was applied to the dorsa of both ears daily for three days. There was no increase in ear swelling 24 hr following the third application at 0.2%-5.0%, indicating that HPT is not an irritant. Cervical (auricular) lymph node cell proliferation was statistically increased (72 hr post-application) at 2% HPT relative to the vehicle control, however, the increase did not reach a sensitization index (SI) of 3. In the MEST, mice sensitized with 1% and 2% HPT and challenged with 2% HPT exhibited significant increases in percent ear swelling, compared to the vehicle irritancy control, at 24 hr, but not at 48 hr, post-challenge. In summary, based on lack of irritancy and on statistical significance in the LLNA and MEST, HPT can be characterized as a sensitizer, but not an irritant.

PS 99 HUMAN LINE1 PROMOTER ACTIVITY IS ENHANCED BY CHEMICAL AND DRUG-INDUCED STRESS IN HEPG2 CELLS.

N. Terasaki, M. Kajikawa and N. Okada. *Department of Biological Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Japan.* Sponsor: J. Sugimoto.

Long interspersed element 1 (LINE1), which is one of retrotransposons, constitutes 17 % of the human genome. 80-100 human LINE1 elements are currently active and can mobilize into a new location of the genome, resulting in alteration of the genomic information. Therefore, active LINE1 elements are considered as a sort of endogenous mutagens. In previous studies, some stresses, for example, heat shock, gamma radiation, UV irradiation and some agents are reported to induce mobilization of retroposons. In this study, to investigate widely what chemicals and drugs have the potential to enhance the LINE1 promoter activity, we established a reporter gene assay system in HepG2 cells using human LINE1. We cloned the 5'-UTR of L1.3, which is one of the most active human LINE1s, in the upstream of a luciferase gene and used it in the assay. The LINE1 promoter activity was measured at 6 and 24 hours after exposure to compounds. We assessed 106 compounds which include anticarcinogenic agents, nonsteroidal antiinflammatory drugs (NSAIDs), hypolipidemic agents, statins and some compounds that induce ER stress, oxidative stress, mitochondrial dysfunction etc. More than 1.5-fold increase of the LINE1 promoter activity was observed by the treatment of 9 compounds (merbarone, benzo(a)pyrene, exo1, iodoacetamide, citrinin, cyclosporine A, fenofibrate, bezafibrate, diflunisal and salicylamide) at both 6- and 24-hour points. Other 12 compounds also elevated the LINE1 promoter activity by 1.2- to 1.5-fold. These results suggest that various toxicants and drug stresses might have the potential to cause genomic mutations in a human body by inducing LINE1 mobilization. Therefore, we should keep in mind the possibility of activation of retrotransposons during drug discovery processes.

PS 100 *IN UTERO* BISPHENOL A EXPOSURE ALTERS METASTABLE EPIALLELE AND GLOBAL DNA METHYLATION PATTERNS IN MOUSE OFFSPRING.

M. S. Nahar, C. Weinhouse, O. S. Anderson, T. R. Jones, S. A. Liberman, L. S. Rozek and D. C. Dolinoy. *Environmental Health Science, University of Michigan, Ann Arbor, MI.*

Genetically identical individuals such as monozygotic twins and inbred mice often display phenotypic discordance, even after controlling for environment. Therefore, epigenetic plasticity has been proposed to play a role in the diverse phenotypes of individuals. Metastable epialleles variably express loci in genetically identical individuals due to epigenetic marks established early in development. Strikingly, these

alleles are especially vulnerable to environmental exposures. The murine viable yellow agouti (A^{vy}) locus is a well-established metastable epiallele that serves as an epigenetic biosensor. Using this model, we previously reported that maternal exposure to bisphenol A (BPA), the monomer forming polycarbonate plastic and epoxy resins, decreases DNA methylation at the A^{vy} locus. Herein, we assess global DNA methylation levels as well as novel murine metastable epiallele methylation patterns in order to identify additional epigenetic loci sensitive to maternal BPA exposure. DNA from d22 liver was isolated from A^{vy}/a and *a/a* mice exposed *in utero* and during lactation to 50 mg BPA/kg diet (N=90) or a control diet (N=76). Global methylation levels were assessed via the Luminometric Methylation Assay, and DNA methylation at candidate metastable epialleles *Dnajb1* and *Glcci1* was analyzed via bisulfite sequencing. Global DNA methylation levels were significantly higher in BPA-exposed mice compared to controls (70.4% and 66.4%, respectively; p-value=0.001). Mean *Glcci1* methylation levels at two intracisternal A particle (IAP) repeats were higher in BPA exposed mice (95.2% and 91.3%) compared to controls (93.7% and 89.9%; p-value= 0.001). No exposure differences were observed in the promoter of *Dnajb1*, which exhibited low levels of methylation in both groups. Both global and site-specific methylation changes may be useful toxicological tools as epigenetic biosensors of environmental exposure and aid in evaluating disease susceptibility.

PS 101 IDENTIFICATION OF CANCER SPECIFIC METHYLATED PROMOTER REGION IN MOUSE BONE MARROW ORIGINATED TUMOR CELLS.

H. Shin[1], S. Jeong[2], J. Kang[1], Y. Park[1], S. Son[1] and H. Kang[1]. [1]*Toxicology & Chemistry Division, NVRQS, Anyang, Republic of Korea and* [2]*GLP Research Center, College of Natural Sciences, Hoseo University, Asan City, Republic of Korea.*

DNA methylation has profound roles in gene regulation and is known as contributing factors in disease and cancer. In cancer cells, abnormal methylation of CpG islands has been widely described. The etiology of bone marrow originated cancer is still largely unknown, but exposure to chemicals has been suggested to be cancer risk factors and associated with the pathologies. The aim of this study is to identify commonly altered methylation genes in mouse bone marrow originated cancer cells compared to normal bone marrow cell for the application of prescreening tool to the bone marrow carcinogenicity of chemicals. Normal bone marrow cells were obtained from 8 weeks old male balb/c mice and 2PK, EL4 and M1 were used as representatives of bone marrow cancer cells. Sheared DNA was immunopricipitated with 5-methylcitosine antibody followed by LM (ligation-mediated) PCR. Amplified DNA were labeled with fluorescence and hybridized to mouse 105K methylation chip (Agilent). Total RNA was isolated from each cell and reverse-transcripted to cDNA. Expression of mRNA was analyzed by real time PCR. Sodium bisulfite treated DNA was amplified and pyrosequencing analysis was conducted using PSQ96MA system. We identified commonly hypermethylated genes such as ABI2, FGF3, BCL11a, STAGE2 and hypomethylated genes like SLMO1 and PNMA2 in promoter regions compared to those of normal bone marrow cells. Hypermethylated genes in promoter region also showed reduced mRNA expression except ABI2 and hypomethylated gene, SLMO1, was represented hyperexpression of its mRNA in cancer cells. To validate the result of BCL 11a, we did pyrosequencing analysis of Bcl11a, which results showed similar pattern of our previous microarray data. We expect that this altered methylation genes could be very useful for diagnosis index bone marrow originated cancers in experimental animals and used as prescreening tools for the bone marrow related carcinogenic evaluation of chemicals.

PS 102 DOSE-DEPENDENT SHIFTS IN A^{VY} COAT COLOR DISTRIBUTION FOLLOWING MATERNAL DIETARY EXPOSURE TO BISPHENOL A.

O. S. Anderson, M. Nahar, T. R. Jones and D. C. Dolinoy. *Environmental Health Science, University of Michigan, Ann Arbor, MI.*

Environmental exposures and nutritional status *in utero* influence epigenetic mechanisms shaping disease susceptibility throughout the life-course. Metastable epialleles are alleles variably expressed due to epigenetic modifications established early in development. The murine *viable yellow agouti (A^{vy})* locus is a metastable epiallele that results in coat color varying from yellow (unmethylated) to pseudoagouti (methylated) due to stochastic methylation of cytosine-guanine dinucleotides among isogenic mice. In yellow mice, constitutive ectopic Agouti expression results in adverse effects including diabetes and adult-onset obesity. Bisphenol A (BPA), a high production-volume chemical in polycarbonate plastic and epoxy resins, is found in consumer products such as food and water containers, receipts, and metal can linings. Utilizing the A^{vy} mouse model, we evaluated dose-dependent maternal dietary BPA exposure and its impact on coat color distribution, an epigenetic biosensor for altered DNA methylation status. At d22, A^{vy}/a offspring of mothers exposed to a diet of 50 mg/kg (n=9 litters), 50 mcg/kg (n=7 litters), or 50 ng/kg (n=8 litters) BPA throughout gestation and lactation were visually rated for coat color status. Replicating previously reported data from our group (Dolinoy et. al. PNAS, 2007), maternal dietary exposure to 50 mg/kg shifts the coat color distribution of Avy/a offspring toward the yellow coat color phenotype compared to offspring of mothers consuming a control corn oil diet (n=11 litters; p-value=0.004). Conversely, maternal dietary exposure to 50 mcg/kg or 50 ng/kg BPA displays a significant shift toward the pseudoagouti coat color phenotype compared to a corn oil control diet (p-value=0.0036, and 0.0001, respectively). Thus far these results suggest BPA alters A^{vy} epigenetic regulation in a non-monotonic fashion, indicating varying exposure levels early in development may modify the epigenome, which consequently influences disease susceptibility throughout life.

PS 103 THE UP-REGULATION OF *AHR* AND *STAT1* GENE EXPRESSION IN LONG-TERM ESTROGEN EXPOSED MCF-7 CELLS: POTENTIAL ROLES OF AUTOCRINE SIGNALING AND EPIGENETIC MECHANISMS.

N. A. Englert[1, 2], B. C. Spink[1] and D. C. Spink[1, 2]. [1]*Laboratory of Molecular Toxicology, Wadsworth Center, New York State Department of Health, Albany, NY and* [2]*Department of Environmental Health Sciences, State University of New York at Albany, Albany, NY.*

The aryl hydrocarbon receptor (AhR) regulates expression of cytochromes P4501A1 and 1B1, enzymes that catalyze metabolism and bioactivation of numerous xenobiotics and endobiotics. Signal transducer and activator of transcription 1 (STAT1) mediates the effects of interferons, regulates expression of immune-response genes, potentiates pro-survival signals, and its elevated expression in tumors appears to provide a growth advantage. We observed that both AhR and STAT1 are up-regulated in long-term estrogen exposed (LTEE) MCF-7 breast cancer cells that were obtained by continuous exposure to 1 nM 17β-estradiol (E2). LTEE cells also showed increased levels of phosphorylated STAT1. To determine the persistence of the up-regulation of STAT1 and AhR expression in LTEE cells, we examined their expression in LTEE cultures after the removal of added E2. Our results indicate that STAT1 expression relies on the continued presence of E2, since total STAT1 levels returned to near control levels after E2 withdrawal, with a half-life of about 9 days. STAT1 expression paralleled the level of activation of the MAP kinase pathway, a known effector of STAT1. Increased steady-state ERK1/2 phosphorylation was observed in LTEE cells, but this decreased after E2 removal. Conversely, the elevated expression of AhR showed no reversion toward control levels after 35 days of E2 withdrawal. The up-regulation of AhR expression was coincident with alterations in histone methylation as determined by chromatin immunoprecipitation, and was reflected in marked changes in AhR-regulated CYP1A1 inducibility. These studies indicate that LTEE alters AhR- and STAT1-regulated processes, and that divergent epigenetic and autocrine signaling mechanisms may be involved in their regulation.

PS 104 EPIGENETIC INACTIVATION OF $P16^{INK4A}$ GENE IS ASSOCIATED WITH RESISTANCE OF GUERIN CARCINOMAS TO DOXORUBICIN AND CISPLATIN.

S. Sarfaraz[1], S. Shpyleva[1], I. Todor[2], N. Yanova[2], V. Chekhun[2] and I. Pogribny[1]. [1]*Biochemical Toxicology, NCTR/FDA, Jefferson, AR and* [2]*Oncology and Radiobiology, R.E. Kavetsky Institute of Experimental Pathology, Kyiv, Ukraine.* Sponsor: V. Tryndyak.

Acquired drug resistance is a major clinical obstacle in the treatment of cancer patients. Therefore, understanding the underlying molecular mechanisms associated with its development is of prime importance. In the present study, we examined the role of epigenetic alterations in transplantable Guerin carcinoma resistant to either Doxorubicin (DOX) or Cisplatin (cisDDP), two cancer chemotherapeutic drugs with different modes of action. Despite this difference, Dox- and cisDDP-resistant Guerin tumors displayed similar alterations in expression of most common cancer-related genes. These changes include down-regulation of *Tp53, Pten, Brca1, and Cdkn2a($p16^{INK4a}$)* genes and up-regulation of *Fos, Vegfa, Vegfc, Plaur, and S100a4* genes. Additionally, both drug-resistant Guerin tumors were characterized by similar epigenetic changes showing pronounced DNA hypermethylation. This was evidenced by the 63% increase in gene-specific CpG island methylation in resistant

tumors as compared to sensitive parental tumors. Gene-specific methylation analysis revealed a profound hypermethylation of the *Cdkn2a(p16^{INK4a})* gene, one of the most down-regulated genes in drug-resistant tumors. The results support the hypothesis that epigenetic changes are important feature of drug-resistant tumors and provide strong evidence of epigenetic alterations development in tumor resistant to different chemotherapeutic drugs.

PS 105 EPIGENETIC ALTERATIONS FOLLOWING DEVELOPMENTAL EXPOSURE TO THE PYRETHROID PESTICIDE DELTAMETHRIN.

A. L. Green[1, 2] and J. R. Richardson[2]. [1]*The Joint Graduate Program in Toxicology, Piscataway, NJ and* [2]*Rutgers University, University of Medicine and Dentistry of New Jersey, The Environmental and Occupational Health Sciences Institute, Piscataway, NJ.*

Previously, we have demonstrated that the offspring of mice exposed to the pyrethroid pesticide deltamethrin (DM) exhibit behavioral alterations and long-term changes in dopaminergic gene expression. However, the mechanism by which these long-term changes occur is not clear. Here, we sought to test the hypothesis that epigenetic alterations were responsible for the changes in dopaminergic gene expression. Pregnant C57BL6 mice were administered vehicle (corn oil) or 3 mg/kg DM every three days throughout gestation and lactation. The offspring were weaned on postnatal day 22, sacrificed at 10-12 months of age and brain regions were harvested. QPCR was performed to determine the expression of the dopamine transporter (DAT), dopamine receptor (D1), and DNA methyltransferases (DNMT)1, 3a, and 3b. DAT expression in the midbrain was significantly increased by 82% in the male offspring of DM-treated mice. D1 expression was increased in the male offspring by 35% and 45% in the striatum and cortex, respectively. Expression of DNMT1, was decreased by 31%, 25%, and 45% in the striatum, cortex, and midbrain, respectively, in the male offspring of DM-treated mice. DNMT3a expression was decreased by 21%, 29%, and 51%, while DNMT3b was decreased by 25%, 44%, and 74% in these same regions. There were no significant changes in DAT, D1 or DNMT expression for female offspring of DM-treated mice. To determine the ability of DNMTs to regulate DAT and D1 expression, DAT-expressing SK-N-AS cells and D1-expressing SK-N-MC cells were treated with the DNMT inhibitor 5-aza-2-deoxycytidine (5-aza). This treatment significantly increased DAT and D1 receptor expression. Taken in concert, these data demonstrate that DNMT regulates expression of the DAT and D1 receptor and that decreased DNMT expression following developmental DM exposure may contribute to the increased expression of the DAT and D1 receptor.
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PS 106 EFFECTS OF CHRONIC EXPOSURE TO 17β-ESTRADIOL ON CELL MORPHOLOGY, GROWTH PATTERN, AND EXPRESSION OF EPIGENETIC REGULATORY GENES.

J. Treas and K. Singh. *The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX.*

Chronic exposure to 17β-estradiol has been shown to be associated with development of benign prostatic hyperplasia and prostate cancer. Estrogens play a role in the regulating the development and function of the prostate at several stages. Studies have shown that estrogens can disrupt expression on a wide range of genes including those involved in carcinogenesis. However, the ability of estrogen to effect expression of genes controlling epigenetic processes has not been fully delved into. Therefore, the objective of this study was to determine the effects of chronic exposure to 17β-estradiol on the expression of genes that regulate the epigenetic process in RWPE-1, a human prostate epithelial cell line. RWPE-1 cells were treated with two concentrations (100pg/mL and 100ng/mL) of 17β-estradiol for 4 months. Cell morphology and growth pattern changes were monitored. To determine the effect of estrogen on the expression of genes that regulate DNA methylation and histone modifications we performed Quantitative Real Time PCR. We also performed western blot to confirm changes in gene expression and histone modifications as a result of estrogen exposure. The result of this study revealed that estrogen exposure causes increased growth and morphological changes in RWPE-1 cells. The gene expression data revealed that estrogen exposure causes slightly decreased expression of histone modifying genes HDAC2, 3, and 7 as well as HMT1, but slightly increased expression of HDAC1 and HAT1. The genes DNMT1, DNMT3a, and MeCP2 all showed slight downregulation, but MBD4 showed a dramatic downregulation depending on concentration. Expression changes in some of the study genes at protein level as well as the changes in histone acetylation and methylation were further confirmed by western blot analysis. This study suggests that chronic exposure to estrogen may cause cancer potentially through altering the expression of epigenetic regulatory genes and histone codes.

PS 107 DISRUPTION OF MITOSIS BY OCHRATOXIN A INVOLVES ALTERED ACETYLATION OF CORE HISTONES.

K. Czakai, W. Dekant and A. Mally. *Department of Toxicology, University of Wuerzburg, Wuerzburg, Germany.*

Ochratoxin A (OTA), a mycotoxin and important food contaminant, is one of the most potent rodent renal carcinogens studied to date. Although controversial results regarding OTA genotoxicity have been published, it is now widely accepted that OTA is not a mutagenic, DNA-reactive carcinogen. Instead, increasing evidence from both in vivo and in vitro studies suggests that OTA may promote genomic instability and tumorigenesis through interference with cell division. The aim of the present study was to provide further support for disruption of mitosis as a key event in OTA toxicity and to understand how OTA mediates these effects. IHKE cells cultured in 8 well microscopy chamber slides were treated with OTA at 0, 1, 5, 10, 25 and 50 μM and monitored by differential interference contrast (DIC) microscopy for up to 16 h. Image analysis confirmed that OTA at concentrations ≥ 5 μM, which correlate with plasma concentrations in rats under conditions of carcinogenesis, causes sustained mitotic arrest and exit from mitosis without nuclear or cellular division. Metaphase chromosomes were characterized by aberrant condensation and premature sister chromatid segregation. These changes were associated with altered phosphorylation and hypoacetylation of core histones, i.e. H3K9ac, H3K18ac, H4K16ac, consistent with the importance of posttranslational histone modifications in determining chromatin structure. To test if OTA directly interferes with histone acetyltransferases (HATs), a class of enzymes which regulate acetylation of histones and non-histone proteins at lysine residues, a HAT activity assay was conducted using total nuclear extracts of IHKE cells. In this assay, OTA significantly blocked HAT activity in a concentration-dependent manner similar to anacardic acid, which was used as positive control, suggesting that OTA may act as an inhibitor of HATs. Overall, results from this study provide further support for a mechanism of OTA carcinogenicity involving interference with the mitotic machinery and subsequent chromosome segregation errors.

PS 108 EPIGENETIC EFFECTS OF CRUDE OIL RECOVERED FROM THE DEEPWATER HORIZON SPILL ON HUMAN KIDNEY CELLS.

H. Kim[1], P. J. Lioy[2], C. P. Weisel[2], R. Portier[3], N. Thakkar[1], J. D. Laskin[2] and D. Heck[1]. [1]*New York Medical College, Valhalla, NY,* [2]*UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ and* [3]*Lousiana State University, Baton Rouge, LA.*

There is increasing concern that human exposures to crude oil from the Deepwater Horizon spill may result in significant toxicity. As a complex mixture of aliphatic, aromatic and heterocyclic hydrocarbons, crude petroleum damages a variety of human organs including the kidney. In the present studies we evaluated the effects of a water-soluble fraction (WSF) of crude spill oil on apoptosis and on antioxidant and inflammatory mediator expression in HEK293 human embryonic kidney cells. WSF was prepared by suspending 1-10 μl of crude oil in 10 ml of cell growth medium (Minimal Essential medium with 10% fetal calf serum) with gentle shaking. Lower doses of oil (<4 μL/mL) had minimal or no effects on cell viability or proliferation. At higher doses (>4 μL/mL), cytotoxicity was evident and DNA fragmentation analysis revealed extensive apoptosis. Using real-time PCR, exposure of cells to the WSF (1 μl/ml, 24 hr) was found to cause a 25-75 fold increase in mRNA of the xenobiotic metabolizing enzymes CYP2E1 and CYP3A, a 4-19 fold increase in the primary antoxidants catalase, manganese superoxide dismutase, and gluthathione peroxidase, and a 7-20 fold increase in the secondary antioxidants glutathione-S-transferase (GST) A1/2, GSTM1 and GSTT1. WSF also caused a 3-50 fold increase in mRNA for enzymes producing inflammatory mediators including inducible nitric oxide synthase and cyclooxygenase-2. Taken together our results indicate that crude oil from the Deepwater Horizon can induce genes important in drug metabolism, cellular antioxidants, and genes mediating inflammatory responses in a kidney epithelial cell line. We speculate that these changes mediate in part toxic insult resulting in tissue injury. These important results will be used as a baseline for testing the relative toxicity of aged crude oil that reached shores in the Gulf region of the US. NIH ES005022, UMDNJ/Rutgers/RW Johnson Hosp Center for Disaster Preparedness and Emergency Response (UCDPER).

PS 109 CD4+ T CELL INTRINSIC TNF RECEPTOR 2 SIGNALING: AN EPIGENETIC TARGET FOR TRANSCRIPTIONAL REGULATION OF IL-2?

J. K. Dey[4], R. S. Schondelmeyer[3], E. N. Sellers[3], P. G. Miller[3], G. Lin[5], M. B. Bonn[4] and S. C. McKarns[1, 2]. *[1]Surgery, University of Missouri School of Medicine, Columbia, MO, [2]Molecular Microbiology & Immunology, University of Missouri School of Medicine, Columbia, MO, [3]Biochemistry, University of Missouri, Columbia, MO, [4]Biological Sciences, University of Missouri, Columbia, MO and [5]Biological Engineering, University of Missouri, Columbia, MO.*

Interleukin (IL)-2 is a key cytokine with pivotal pleotropic regulatory roles in maintaining immune homeostasis and peripheral tolerance. Thus, a mechanistic understanding of IL-2 production is central to our understanding autoimmunity, chronic inflammatory disorders, and cancer. Histone modifications, DNA cytosine methylation, and transcription factor occupancy regulate chromatin remodeling and promoter activity of the IL-2 gene in CD4+ T cells. We have previously demonstrated that tumor necrosis factor-alpha (TNF)receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) double knockout mice produce less IL-2, and this associates with reduced c-Rel binding to the proximal IL-2 enhancer/promoter. TNF has dual proinflammatory and immunoregulatory properties with key physiological and pathological regulatory roles, and the differential usage of its receptors has been proposed to contribute to its opposing biological activities. The objective of this study was to define the TNF receptor(s) responsible for modulating IL-2 production. CD4+ T cells from TNFR1, TNFR2, and TNFR1/2 double knockout IL-2 GFP knock-in mice were stimulated and compared with control CD4+ T cells from wild-type B10.A mice. The frequency of IL-2 producers and a delay in the onset of IL-2 transcription was observed in the TNFR2 and TNFR1/2-deficient T cells only. Moreover, stimulation of purified CD4+ T cells with plate-bound anti-CD3 and anti-CD28 demonstrated a T cell intrinsic regulatory role for TNFR2 on IL-2 promoter activity. Given that c-Rel is essential for chromatin remodeling of the IL-2 enhancer/promoter, our results suggest that TNFR2 signaling may facilitate epigenetic regulation of cytokine production.

PS 110 EPIGENETIC MODULATION OF XENOESTROGEN-INDUCED BREAST CANCER.

L. Wang, W. Jiang, B. Moorthy and S. R. Kondraganti. *Department of Pediatrics, Baylor College of Medicine, Houston, TX.*

Dietary phytochemicals inhibit cancer formation by multiple mechanisms. These mechanisms include epigenetic changes resulting from the inhibition of histone deacetylase (HDAC) activity. HDACs affect histone acetylation status and results in cell cycle arrest and/or apoptosis. Targeting the epigenome, including the use of histone deacetylase (HDAC) inhibitors, is a novel strategy for cancer chemoprevention. Sulforaphane is an isothiocyanate found in cruciferous vegetables, such as broccoli and broccoli sprouts. Exposure to xenoestrogens leads to adverse health effects and have a potential effect on oncogenes, specifically in relation to breast cancer. 3-Methyl-cholanthrene (3-MC) represents a novel subclass of xenoestrogenic compounds that activates both ER-α and AhR signaling pathways. In the present study, we investigated the role of sulforaphane in inhibition of xenoestrogen induced cancer formation. Growing evidence show that sulforaphane acts through different chemoprotective mechanisms. To understand the anticarcinogenic potency of sulforaphane, we studied induction of phase 2 detoxification enzymes along with its ability in inhibition of HDAC activity. The wild type (C57BL/6J) female mice were exposed to 3-MC and sulforaphane, alone and in combination. Significant reduction of phase I enzymes along with elevation of several phase II enzymes were observed in hepatic and mammary tissues of female mice treated with sulforaphane. Thus, sulforaphane could modulate the Phase I/Phase II enzymatic systems, a critical mechanism involved in the formation of cancers. Our results also show the differential expression of receptors on exposure to environmental estrogens. Our studies show that sulforaphane acted as an HDAC inhibitor in the xenoestrogen exposed rodent hepatic and mammary tissues. This results in enhanced histone acetylation, derepression of P21 and Bax, leading to cancer prevention. Our results show the ability of dietary phytochemicals to reactivate epigenetically-silenced genes in cancer cells and thus have important implications as epigenetic modulators in cancer prevention and therapy.

PS 111 THE ROLE OF NICOTINE IN THE INDUCTION OF GENOMIC INSTABILITY IN BREAST CELLS.

C. L. Clemens and J. W. DuMond. *Environmental Science and Technology, Texas Southern University, Houston, TX.*

Studies in the United States have indicated that active and passive smoking are associated with increased breast cancer risk. However, there is little direct evidence of a direct effect of environmental tobacco smoke in breast carcinogenesis. This study examines the effects of nicotine on the proliferation, gene regulation and ability to induce oxidative damage in MCF-7 breast cancer cells, as we postulate here that nicotine can produce genomic instability in breast cancer cells. Cell proliferation studies were preformed on MCF-7, a human breast cancer cell line. Cell proliferation studies were performed at concentrations of 1ng/ml, 10ng/ml, and 100pg/ml of nicotine hydrogen tartrate salt (purity, ≥98%). Cells were counted after 72 hours of exposure to either treatment or control. The expression of critical DNA repair and DNA damage genes was analyzed by Real-Time Reverse Transcriptase Polymerase Chain Reaction after acute exposure to nicotine. Increased oxidative stress was measured by Trevigen's Fragment Length Analysis using Repair Enzymes (FLARE®) assay. Cell proliferation studies showed a significant increase in cell growth at 10ng/ml (25%) and at 100 pg/ml (20%) when compared to control cells. RT-PCR showed a down regulation in gene expression of important regulatory genes, such BRCA1, EXO1, PRKDC, and other DNA damage repair genes such as; MRE11A, N4BP2, NBN, PCNA, PMS2, RAD17, and XPA. Exposure also resulted in an up regulation of gene expression in important cell regulating genes; DDB1, MAPK12, GADD45G and PPP1R15A, IGHMBP2, TP73 and PCBP4, and LIG1 up regulation were also detected. Other DNA damage repair genes such as; BTG2, CIDEA, ERCC2, FANCG, FEN1, MPG, PPP1R15A, SEMA4A, TREX1, XRCC1, and XRCC3 also had a significant up regulation. Oxidative damage repair genes such as MUTYH, OGG1 and NTHL1 were significantly up regulated leading to the use of the FLARE assay using the Fpg enzyme to further confirm oxidative damaged caused by nicotine. The results of the FLARE assay confirmed that Nicotine (10 ng/ml) exposure increases oxidative damage in MCF-7 cells.

PS 112 INDUCTION OF BASE EXCISION REPAIR ENZYMES APE1 AND NTH1 IN RAT SPLEEN FOLLOWING ANILINE EXPOSURE.

H. Ma, J. Wang and M. Khan. *Pathology, University of Texas Medical Branch, Galveston, TX.*

Mechanisms by which aniline exposure elicits splenotoxic response, especially the tumorigenic response, are not well-understood. Earlier, we have shown that aniline exposure leads to oxidative DNA damage and up-regulation of OGG1 and NEIL1/2 DNA glycosylases in rat spleen. However, contribution of apurinic/apyrimidinic endonuclease 1 (APE1) which plays a central role in the repair of oxidized and alkylated bases and endonuclease III homolog 1 (NTH1) which is capable of removing oxidized pyrimidines, such as thymine glycol (Tg) from DNA via the base excision repair (BER) pathway, in the repair of aniline-induced oxidative DNA damage in the spleen is not known. This study was, therefore, focused on examining if APE1 and NTH1 also contribute to the repair of oxidative DNA lesions in the spleen, in an experimental condition preceding a tumorigenic response. To achieve this, male SD rats were subchronically exposed to aniline (0.5 mmol/kg/day via drinking water for 30 days), while controls received drinking water only. By quantitating the cleavage products, the BER activity of APE1 and NTH1 were assayed using substrates containing tetrahydrofuran and Tg, respectively. Aniline treatment led to significant increases in APE1- and NTH1-associated BER activity in the nuclear extracts of spleen compared to the controls. Western blot analysis showed that protein expression of APE1 and NTH1 in the nuclear extracts of spleen from aniline-treated rats was 2.7- and 1.9-fold higher than controls, respectively. Likewise, real-time PCR analysis for APE1 and NTH1 mRNA expression in the spleen showed 3.2- and 2.9-fold increases, respectively, in aniline-treated rats compared to controls. Aniline treatment also led to stronger immunoreactivity for both APE1 and NTH1 in the spleens, confined to the red pulp areas. These results, thus, show that aniline exposure is associated with an induction of APE1 and NTH1 in the spleen. The increased repair activity of APE1 and NTH1 could be an important mechanism for the removal of oxidative DNA lesions. Supported by NIH ES06476.

PS 113 ENHANCED NUCLEOTIDE EXCISION REPAIR OF COMBINED 17ALPHA-ETHINYLESTRADIOL AND UV IN ZEBRAFISH LIVER CELLS.

S. Tang[1], E. G. Notch[2], V. Allagadda[1] and G. D. Mayer[1]. *[1]The Institute of Environmental and Human Health, Texas Tech University, Lubbock, TX and [2]Department of Physiology, Dartmouth Medical School, Hanover, NH.*

Ultraviolet (UV) radiation damages cellular molecules, and has been indicated to induce nucleotide excision repair (NER) to remove DNA lesions including cyclobutane pyrimidine dimmers (CPDs). Recent studies suggest that exposure to 17alpha-Ethinylestradiol (EE2) decreases mRNA abundance of key NER genes (XPC and XPA) and inhibits cellular NER repair capacity in zebrafish (Danio rerio) liver (ZFL) cells. However, the impact of co-exposure of UV light and EE2 on NER is still unknown. The aim of this study was to evaluate the combined effects of EE2

exposure and UV radiation on DNA repair system of ZFL liver cells, a highly competent species in terms of damage repair triggered by UV irradiation. Here we show UV affected viability of ZFL cells in a dose dependent manner, while EE2 (from 1 to 100 nM) did not alter viability beyond the UV. For gene expression analysis and DNA damage repair capacity assay, ZFL cells were co-exposed to 20 J/m2 UV and different concentrations of EE2. The early response observed (2 h) showed decrease in the mRNA abundance of XPC gene, while the late response observed (6 and 24 h) induction of the XPA gene. Time-course of DNA adduct (CPDs) amounts corresponded to the transcriptions level of XPC and XPA gene. Co-exposure of EE2 and a complete of estrogen receptor antagonist (ICI 182,780) also lead to reduced NER capacity at 2 h after 20 J/m2 UV radiation, but enhanced NER capacity at 24 h. Taken together, NER can be induced in ZFL cells by the combination of UV radiation and EE2 exposure in a time and EE2-concentration dependent manner and this effect can also happen with the presence of estrogen receptor antagonist, indicating that EE2 can affect NER process not via the activation of nuclear estrogen receptor.

PS 114 HOMOLOGOUS RECOMBINATION IN THE CELLULAR RESPONSE TO SULPHUR MUSTARD.

P. A. Jowsey, F. M. Williams and P. G. Blain. *Medical Toxicology Centre, Newcastle University, Newcastle Upon Tyne, United Kingdom.*

Sulphur mustard (SM) is a vesicant that causes damage to the skin, eyes, respiratory system and bone marrow. To develop treatments, it is necessary to have a detailed understanding of the cellular/biochemical changes induced by SM, as well as the mechanisms that cells employ to protect against SM toxicity. SM is able to react with DNA, forming DNA monoadducts and crosslinks. DNA crosslinks are cytotoxic lesions, normally repaired by a pathway involving components of nucleotide excision repair and homologous recombination (HR). This study aimed to investigate the role of HR in the response to SM and investigate whether activation of HR protected against SM toxicity. The toxicity of SM in HR-deficient cells was investigated. VC8 cells (HR-deficient) and VC8-B2 cells (HR-proficient) were treated with SM (0.1μM - 2μM) for 48 hours before measuring cell viability using an MTS assay. At each dose, VC8 cells were significantly more sensitive to SM than VC8-B2 cells. The greatest difference was after 2μM SM, at which dose VC8-B2 cells had a relative viability of approximately 90% compared to just 30% in VC8 cells. Similar results were obtained using Hela cells, depleted of BRCA2 (and thus HR-deficient) using siRNA. As a further marker for HR, the formation of RAD51 nuclear foci was investigated in VC8-B2 and Hela cells using immunofluorescence. A dose-dependent increase in levels of RAD51 foci was observed, confirming the role of HR in the response to SM. A recent study described a compound (RS-1) that was able to promote HR by increasing the DNA binding activity of RAD51. In the present study, RS-1 was added to Hela or TK6 cells prior to treatment with SM. After 48h, cell viability was measured. RS-1 increased cell survival after SM. The maximum protection observed was a 30% increase in cell survival in TK6 cells and a 65% increase in Hela cells. This study has demonstrated that HR is involved in the cellular response to SM and that manipulation of this pathway reduces cellular toxicity and may offer therapeutic possibilities.

PS 115 ARSENIC INHIBITS THE NUCLEOTIDE EXCISION REPAIR PATHWAY IN HUMAN LUNG CELLS AND MOUSE KERATINOCYTES.

N. Holcomb, M. Goswami, T. Scott, T. Bhattarai, J. D'Orazio, D. Orren, G. Gairola and I. Mellon. *Graduate Center for Toxicology, University of Kentucky, Lexington, KY.*

Chronic exposure to arsenic has been linked to several types of cancers in humans including skin and lung cancer. However, the mechanisms underlying its role in cancer are not well understood. It has been proposed that exposure to arsenic enhances the carcinogenicity of UV light in producing skin cancers and the carcinogenicity of tobacco smoke in producing lung cancers. UV light and components of tobacco smoke produce different types of DNA damage that are removed by the nucleotide excision repair pathway (NER). The removal of DNA damage by NER reduces the mutagenicity and carcinogenicity of UV light and tobacco smoke. The aim of the present study was to determine if arsenic inhibits NER in primary human lung fibroblasts and mouse keratinocytes. Cells were exposed to sodium arsenite or mock-treated for 24 h and then irradiated with UV-C. UV introduces 2 major DNA lesions; cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4 PPs). Cells were harvested immediately after UV irradiation (0 h) or after incubation for additional periods of time to monitor removal of 6-4 PPs using antibodies specific to 6-4 PPs in a slot blot assay. The amount of 6-4PPs decreased rapidly in untreated cells but persisted significantly longer in cells treated with sodium arsenite. Thus, the removal of 6-4PPs in human lung fibroblasts and mouse keratinocytes was inhibited by arsenic in a dose-dependent manner. XPC is essential for DNA damage recognition in NER. Treatment of lung fibroblasts and mouse keratinocytes with sodium arsenite significantly reduced the abundance of XPC protein. The abundance of XPC RNA was greatly reduced in human lung fibroblasts treated with arsenic. The inhibition of NER by arsenic may reflect a novel mechanism underlying the role of arsenic exposure in enhancing cigarette smoke induced lung carcinogenesis and UV light induced skin cancer. (Supported in part by the Kentucky Lung Cancer Research Program).

PS 116 EXPLORING THE ROLE OF NUCLEOTIDE EXCISION REPAIR (NER) AND HELICASE IN ZIDOVUDINE (AZT)-INDUCED GENOTOXICITY.

D. Momot, T. A. Nostrand, K. John, M. C. Poirier and O. A. Olivero. *LCBG, National Cancer Institute, Bethesda, MD.*

Zidovudine (AZT), a DNA replication chain terminator, is an antiretroviral nucleoside reverse transcriptase inhibitor (NRTI) that induces genotoxic effects that include micronucleus (MN) formation, and centrosomal amplification (CA, >2 centrosomes /cell). In this study we cultured mesenchymal cells obtained from bone marrow of C57BL/6J mice that were either wild type (WT), or deficient in NER (Xpa-/-) or a helicase gene BLM (known as Bloom Syndrome). To explore the role of Xpa, a gene controlling initiation of NER, in processing of AZT-induced genotoxic damage, cultured cells were exposed to 10 and 100 μM AZT for 24 hr, and stained immunohistochemically. MN formation was revealed by DAPI staining. In WT cells exposed to 0, 10, 100 μM AZT, there were 3.39, 3.60, and 5.31% of cells with MN, compared to 15.20, 16.49, and 15.04% for the same AZT doses in Xpa-/- cells. CA was localized by anti-pericentrin staining. In WT cells exposed to 0, 10, and 100 μM AZT, 12.2, 15.2, and 19.7% of cells showed CA, respectively, and in Xpa-/- cells there were 18.0, 28.7 and 33.9% of cells with CA, respectively. In summary, both aneuploidy and CA were found in mouse mesenchymal cells exposed in culture to AZT. Thus, the presence of an intact NER system appears to protect cells against genoxicity induced by AZT exposure. Repair of AZT-induced DNA damage is considered to occur through the Base Excision Repair pathway, but these experiments suggest that NER also plays a role. To analyze the role of BLM in protection against AZT incorporation, the same standard protocol was followed. While CA was not significant, in vitro exposure to 0, 10, 100 and 200 μM AZT for 24 hr showed 0.84, 1.20, 1.78, and 1.51% of WT cells with MN, while BLM cells had 2.7, 6.11, 10.28, and 13.04% of cells with MN. Although more samples of DNA repair deficient syndromes are being analyzed, the data suggest that intact helicase is required for protection against AZT-induced genotoxicity.

PS 117 DOSE-RESPONSE EVALUATION OF P53 AND H2AX DNA DAMAGE RESPONSE BY METHYL METHANESULFONATE, ETOPOSIDE, AND QUERCETIN.

B. Sun[1], K. Daniels[1], S. Ross[1], Q. Zhang[1], M. Andersen[1], P. Carmichael[2] and R. Clewell[1]. [1]*The Hamner Institute, Durham, NC and* [2]*Unilever SEAC, Bedfordshire, United Kingdom.*

When cells encounter DNA damage, they respond by initiating cell cycle arrest, damage repair or apoptosis. How DNA damage pathways differentially respond to diverse DNA damage is not yet fully understood. The current study used the HT1080 human fibrosarcoma cell line, which expresses a wild-type p53, to examine time- and dose- dependent response of H2AX phoshporylation (p-H2AX), total p53 and p53 phosphorylation (p-p53), with exposure to methyl methanesulfonate (MMS; methylating agent) etoposide (ETP; DNA/topoisomerase II binding chemotherapeutic) and quercetin (QUE; oxidative flavonoid). We found that 1 to 1000 μM MMS, 0.1 to 100 μM ETP, 1 to 100 μM QUE dose-dependently decreased HT1080 cell viability. Analysis of cell cycle by high throughput bioimaging demonstrated significant G0/G1 arrest at 10 μM QUE, while ETP induced M phase arrest at 1 μM. MMS, however, induced a G2/M arrest only at 10000 μM. Bioimaging and immunoblots showed that both ETP and QUE caused time- and dose-dependent increases in the DNA double strand break marker p-H2AX within 24 hr, while MMS had a marginal effect. ETP showed greatest ability to induce p53 and p53 phosphorylation at ser15 (p-p53), while MMS had the weakest effect. Results with this cell line are being compared to the lymphoblast AHH-1 cell line, which has been used in mutagenesis studies, in order to compare the dose-dependence of cellular repair response and increased mutation frequency. The preliminary data for AHH-1 cells suggest that AHH-1 cells, albeit less sensitive to those reagents, show comparable p53 and p-H2AX DNA damage response to HT1080 cells. Collectively, these results suggest that ETP and QUE but not MMS cause DNA double strand breaks and those chemicals differentially activate DNA damage repair pathways.

PS 118 TO STUDY THE SIGNAL TRANSDUCTION ON 2-ABP & 4-ABP INDUCED DNA DAMAGE IN HEP G2 CELL.

J. Wong[1], J. Wu[2], S. Chen[1] and L. Chen[3]. [1]*Department of Biotechnology, National Kaohsiung Normal University, Kaohsiung, Taiwan,* [2]*Department of Applied Chemistry, Fooyin University, Kaohsiung, Taiwan and* [3]*Department of Medical Nutrition, I-Shou University, Kaohsiung, Taiwan.*

The International Agency for Research on Cancer declared that 4-aminobiphenyl (4-ABP) was carcinogenic to human. 2-aminobiphenyl (2-ABP) is its analogue. Our previous results showed that 4-ABP and 2-ABP were examined for their ability to induce oxidative DNA damage in Hep G2 cells. However, the molecular and cellular mechanisms underlying 2-ABP & 4-ABP associated genotoxicity are not fully understood. Our goal in this study was to determine the DNA damage signaling pathway. We found that 2-ABP and 4-ABP could induce γ-H2AX phosphorylation, a sensitive DNA damage marker, in Hep G2 cells, suggesting that DNA damages were elicited by 2-ABP and 4-ABP. Further, Hep G2 cell were incubated with 200 μM 2-ABP and 200 μM 4-ABP for various periods, that induced the activation of mitogen-activated protein kinase family (MAPK) proteins including JNK and ERK 1/2 were detected in 1h. In addition, we found 2-ABP treatment inhibited cell growth by inducing G0/G1 phase arrest. Whereas we suggest that 2-ABP and 4-ABP could induce DNA damage and activation MAPK phosphorylation but 2-ABP can only induced cell cycle arrest. Our findings suggested that the mechanisms of induced DNA damage in Hep G2 cell by 2-ABP and 4-ABP were different.

PS 119 GENOTOXICITY AND CHANGES IN GENE EXPRESSION PROFILES INDUCED BY AIRBORNE PARTICLES IN HUMAN CELL LINES.

J. Topinka, K. Uhlirova, H. Libalova, A. Milcova, J. Schmuczerova and R. J. Sram. *Genetic Ecotoxicology, Institute of Experimental Medicine AS CR, Prague 4, Czech Republic.*

Human embryonic lung fibroblasts (HEL) and human alveolar adenocarcinoma cells A549 (model of human lung epithelial cells) were employed to assess the genotoxicity induced by complex mixtures of organic air pollutants adsorbed onto respirable air particles (PM2.5) collected by high volume samplers in 4 localities of the Czech Republic (Ostrava-Bartovice, Ostrava- Poruba, Karvina a Trebon) differing by the extent of the air pollution. To get more insight into the mechanisms of the toxic effects, changes in gene expression profiles caused by organic extracts from PM2.5 particles in HEL and A549 cells were investigated. For this purpose, DNA adduct forming activity of extractable organic matter (EOM) from the PM2.5 particles was analyzed by 32P-postlabelling at subtoxic EOM concentrations of 10, 30, and 60 ug/ml. Simultaneously, whole genome expression profiles using Illumina reader and Human HT-12 v2 Expression BeadChip Kit were determined. For both HEL and A549 cells, dose dependent increase of DNA adduct levels was found. Comparable adduct levels were induced by various EOMs in both cell types. Close correlation between DNA adduct levels induced by all EOMs in both cell lines and c-PAHs concentrations in EOMs suggests that genotoxicity of the polluted air is much closely related to c-PAHs content than to PM2.5. The results further suggest that ferrous metallurgy and coke oven emissions in the proximity of Ostrava-Bartovice are much stronger sources of genotoxic compounds forming DNA adducts than traffic emissions in Ostrava-Poruba (3-fold higher traffic density). First analysis of gene expression data suggests that multiple genes were deregulated by EOM by dose-dependent manner. Biological processes (metabolic and signaling pathways) affected by organic air pollutants bound on PM2.5 were identified in Ostrava-Bartovice (polluted mostly by industrial emissions) and in Ostrava-Poruba (mostly traffic related air pollution). Supported by the Czech Ministry of Education (grant #2B08005) and by AS CR (grant AV0Z50390512).

PS 120 MOLECULAR DETERMINANTS OF DNA POLYMERASE ETA FIDELITY.

S. C. Suarez and S. D. McCulloch. *Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.*

Y-family DNA polymerases are required to bypass lesions encountered during DNA replication. This bypass prevents replication fork collapse and DNA strand breaks. UV light can cause not only cyclobutane pyrimidine dimers (CPD), but also oxidative damage such as 7,8-dihydro-8-oxo-guanine (8-oxo-G). 8-oxo-G is a lesion that is generated thousands of times per day in each human cell under normal conditions as a consequence of cellular respiration. DNA polymerase eta (pol eta) is able to bypass a number of lesions that can block replication fork progression, including CPD and 8-oxo-G. Recent reports of pol eta crystal structures complexed with CPD show a more open active site, as well as implicating a number of active site residues that play a role in the bypass of CPD. To investigate the role of these residues in the bypass of 8-oxo-G, we have created and purified a set of single amino acid substitution mutants of pol eta. We used these mutants to test the effect of these residue changes on ability of the polymerase to bypass the lesion at template to enzyme ratios and time points that approximate a lesion bypass event in vivo. We also used an assay to examine the effect of these mutants on the insertion fidelity of pol eta past 8-oxo-G. Our results indicate that these residue changes can alter both the fidelity and bypass of the enzyme. Some changes can confer a mutator phenotype, while others have an opposite effect. These changes can also reduce the ability of the polymerase to bypass 8-oxo-G. These results indicate that pol eta walks a fine line between the ability to bypass lesions while still maintaining adequate replication fidelity. The increase in bypass at the cost of decreased fidelity has implications for an organism at multiple levels. The characterization of these mutants gives us insight as to the molecular determinants for the most important properties of pol eta in performing its function of bypassing lesions and preventing mutation during lesion bypass.

PS 121 MECHANISMS OF THE WERNER SYNDROME PROTEIN IN PROTECTING AGAINST CR(VI) INDUCED TELOMERE LOSS.

F. Liu, K. E. Knickelbein, A. Barchowsky and P. L. Opresko. *Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA.*

Werner syndrome protein (WRN) is a DNA helicase-exonuclease that facilitates telomeric DNA replication. Telomeres consist of guanine-rich DNA repeats coated by specialized proteins that protect chromosomal ends from being processed as DNA breaks. Disruption of telomere replication can lead to dysfunctional telomeres, which contribute to aging related diseases and cancer. However, limited information is available regarding the effects of environmental exposures on telomere stability. Hexavalent chromium (Cr(VI)) is an established environmental risk factor for lung cancer in the occupational setting. We showed previously that WRN suppresses Cr(VI)-induced telomere loss and are currently investigating the mechanism. Since Cr(VI) induces adducts that arrest DNA polymerases predominantly in contiguous guanine runs, we predict that these lesions block telomere replication. We found Cr(VI) caused oxidative stress only at high levels (>100 μM), eliminating this as a mechanism for telomere loss. Cr(VI) increased foci formation of polymerase eta, which synthesizes past DNA lesions that block replicative polymerases. Thus, Cr(VI)-induced polymerase eta localization at telomeres serves as a marker for telomeric replication blocking lesions. We found WRN deficiency resulted in delayed reinitiation of Cr(VI)-induced stalled replication forks. We are using chromosome-orientation fluorescent in situ hybridization to examine WRN roles in repairing Cr(VI)-disrupted replication forks at telomeres by homologous recombination. Furthermore, we are testing WRN roles in suppressing Cr(VI)-induced DNA deletions and mutagenesis in telomeric DNA using a shuttle vector system harboring the supF gene and telomere repeats as a mutagenic target. Our study indicates that Cr(VI) generated DNA replication but not oxidative stress contributes to telomere loss at occupationally relevant levels and that WRN facilitates repair of Cr(VI)-disrupted telomere replication.

PS 122 ANALYSIS OF MOLECULAR SPLINT MUTANTS OF HUMAN DNA POL η AND THEIR EFFECT ON POLYMERASE PROPERTIES.

R. A. Beardslee and S. D. McCulloch. *Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.*

DNA polymerase η (Pol η) is responsible for the bypass of cyclobutane pyrimidine dimers (CPDs) during DNA replication as well as other lesions such as 8-oxoguanine (oxoG). Both are ubiquitous lesions; the former is produced by exposure to UV radiation, while the latter is generated by reactive oxygen species (ROS) yielded during xenobiotic metabolism in addition to normal cellular function. It follows that Pol η is indispensable to successful completion of DNA replication and organism survival. It has been recently reported that a β-strand (amino acids 316-324) in the little finger region of Pol η appears important to correctly align the template strand with the catalytic core of the enzyme. We hypothesized that modification of these residues would interfere with correct enzyme-DNA alignment and alter Pol η activity and fidelity. To study the role of these mutations, we expressed catalytic core amino acids 1-511 of human Pol η in *E. coli*. We generated both wild type enzyme as well as enzyme with single amino acid substitutions at residues Pro-316, Thr-318, Gly-320, Ser-322 and Asn-324. Overexpressed protein was purified by chromatography using HiTrap™ Chelating HP (GE) with subsequent application of Pol η rich fractions to Mono S™ (GE). Purified protein fractions and DNA

oligomers synthesized with both CPD and oxoG lesions were then used in *in vitro* assays to evaluate both polymerase activity and fidelity during replication of undamaged and damaged DNA. We anticipate that some or all of these β-strand mutations will alter the activity and fidelity of Pol η compared to wild-type and that the results from these studies will suggest that these amino acids provide an important structural component to the enzyme necessary for the successful bypass of DNA lesions during replication as well as organism survival. Furthermore, we believe that the findings will contribute to an explanation for the low bypass fidelity of Pol η past oxoG lesions.

PS 123 COMMONALITY AND STOCHASTICITY IN GENE EXPRESSION PROFILES DURING AGING PROCESS.

T. Inoue[1] and Y. Hirabayashi[2]. [1]*Center for Biological Safety & Research, National Institute of Health Sciences, Tokyo, Japan and* [2]*Division of Cellular & Molecular Toxicology, Center for Biological Safety & Research, National Institute of Health Sciences, Tokyo, Japan.*

Senescence is conceptually considered to be xenobiotic responses to "time", where essential biological responses during aging may be based on a lifetime-dependent relationship between living creature and xenobiotic materials in relationship with oxidative stresses and photo-stimulation, among others. When one examines a hundred senescent mice, senescent phenotypes may be generally observed nearly in all mice. Among them, there may be common changes in the most on one hand, there may be also probabilistic phenotypes observed individually in case by case manner. When one compares those phenotypes in senescent mice comparing with those of less senescent young mice by gene chip microarray, two different categories of gene expression profiles are recognized; i.e., one corresponds to common aging profiles (CAPs) selected by two-way analysis of variance (ANOVA) and the latter corresponds to stochastic aging profiles (SAPs) selected by principal component analysis (PCA). Reason we focused such stochastic gene expression was because we recognized that gene expression profiles in microarray were mostly uniform (common) when those in RT-PCRs were uniform, whereas those profiles in microarrays individually vary (stochastic) when those data in RT-PCRs were divergent individually. CAPs are definitive expression profiles to senescent mice whereas SAPs are probabilistic expression profiles observed in one senescent mouse to the other. Gene expression profiles compared bone marrow cells from 2- and 21-month-old male mice of the C3H/He strain provided 122 probe sets of CAPs and 1005 of SAPs, resp.. When one examines the relationships between CAP-related and SAP-related signalings using network database, stochastic gene expressions scattered in the networks occasionally merged downstream toward the CAP genes, stochastically. CAPs seem to be conciliatorily regulated by positive and negative signals from various stochastic genes differing from one case to another.

PS 124 COMPARISON OF TOXICOGENOMICS PROFILES TO DISCOVER CO-REGULATED GENES.

Y. Igarashi[1], N. Nakatsu[1], H. Yamada[1], A. Ono[2], Y. Ohno[2] and T. Urushidani[1, 3]. [1]*National Institute of Biomedical Innovation, Ibaraki-City, Osaka, Japan,* [2]*National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan and* [3]*Doshisha Women's College of Liberal Arts, Kyotanabe-City, Kyoto, Japan.*

We have developed in silico methodology for toxicogenomics data to compare a pair of probe sets considering with their time courses and dose effects. This method can be applied to any kinds of gene expression data which is obtained in a systematic manner. The toxicogenomics project in Japan, started in 2002, produced genome-wide gene expression data of the liver of rat in vivo and vitro using 150 pharmaceutical drugs. The gene expression data has been obtained at 8 time points and 4 dose levels in vivo, and 3 time points and 4 dose levels in vitro. One of the reasons the identification of safety biomarkers is difficult is that transcriptional responses following treatment are embedded in the feed back loops of signaling pathways. Those responses would be varied at different time points and dose levels even if same drug is treated. Therefore, it is high opportunity to identify co-regulated genes when the expression levels are similar at continuous time points and dose levels. We have developed simple and flexible algorithm to compare genome-wide transcriptional profiling consisting of multiple time and dose points. Our method compares gene expression profiles between a pair of probe sets. The similarity is calculated using a pair of vectors based on the expression levels at continuous 2 time points and 2 dose levels. For example, a probe which consists of 8 time points and 4 dose levels has 21 vectors for the comparison. Using this method, we have estimated clusters of co-expressed gene sets including several known co-regulated genes by treatment of lipopolysaccharide. This method has a great potential to identify co-regulated genes which lead to identification of safety biomarkers.

PS 125 USE OF PATHWAY ANALYSES TO IDENTIFY POTENTIAL TARGET SAFETY RISKS FOR ANTIBODY DRUG CONJUGATES (ADC).

B. Lu[1], L. Obert[1], A. Hooper[2] and M. Gosink[1]. [1]*Drug Safety Research & Development, Pfizer, Groton, CT and* [2]*Center for Integrative Biology & Biotherapeutics, Pfizer, Pearl River, NY.*

Antibody drug conjugates (ADC), which use an antibody to deliver a cytotoxic drug selectively into a target cell, represent a new and powerful therapeutic approach against diseases such as cancer. Different from a traditional drug's mode of action (MOA), an ADC achieves efficacy largely by selectively eliminating target-expressing cells. Assessing the target safety for an ADC program at a early development stage (e.g. pre-lead development) would be difficult because there is a lack of in vivo as well as in vitro assays to experimentally approach such issues. Here we present an in silico approach to use pathway analyses to identify potential target safety risks for Antibody drug conjugates (ADC). We propose that the primary target safety risks for ADC should first be analyzed at the cellular level; essentially the target-expressing cell should be approached as an integral unit. Secondary risks should be evaluated using knowledge of pathway databases to study the ADC target and its downstream pathways. Because ADC therapy can remove all cells expressing a particular target, any extracellular signaling performed by target expressing cells would also be ablated. The absence of those signaling molecules could have a significant impact on neighboring cells as well as other tissues. We introduced such a working concept by using Ingenuity pathway analysis software on two ADC targets CD19 and CD30. We further ran the analysis on a hypothetical ADC target: EGFR and found target risk for EGFR ADC therapy may be greater than those risks of conventional anti-EGF therapy because in conventional anti-EGF therapy feedback mechanism could partially compensate for the loss of EGF. Given the huge interest and investment in ADCs, the knowledge driven approach presented here might help to highlight some potential target safety risks in their early development stage.

PS 126 COMPARISON OF NEXT GENERATION SEQUENCING AND MICROARRAY ANALYSES OF GENE EXPRESSION IN KIDNEY OF THE RATS TREATED WITH CARCINOGEN ARISTOLOCHIC ACID.

T. Chen[1], Z. Li[1], Z. Su[2] and L. Shi[3]. [1]*Division of Genetic and Molecular Toxicology, U.S. FDA-NCTR, Jefferson, AR,* [2]*Z-Tech, U.S. FDA-NCTR, Jefferson, AR and* [3]*Division of System Biology, U.S. FDA-NCTR, Jefferson, AR.*

Next-generation sequencing (NGS) has been used to evaluate gene expression level. In this study, we compared gene expression profiles of aristolochic acid (AA) in rat kidney generated from NGS and microarray technologies. AA is the active component of herbal drugs derived from Aristolochia species that have been used for medicinal purposes since antiquity. AA, however, induced nephropathy and urothelial cancer in humans and malignant tumors in the kidney, urinary tract and other tissues in rodents. Rats were treated with 10 mg/kg or vehicle control for 12 weeks and the kidney tissues were quickly isolated and frozen immediately after the treatment. Four samples for the treatment and 4 for the control were used for the comparison. The transcriptome analysis of the samples was conducted using the Affymetrix Rat Genome 230 2.0 Microarray for the microarray method and Illumina Genome Analyzer II for the NGS approach. The gene expression data generated from both platforms were processed using Significance Analysis of Microarray (SAM) for determining the deferentially expressed genes (DEG) and Ingenuity Pathway Analysis for defining the altered biological functions. The gene expression profiles resulted from both platforms showed that AA treatment produced a large number of DEGs, 1297 for microarray and 4247 for NGS. There were 925 DEGs that were commonly identified by both platforms. Functional analysis demonstrated that the major biological processes dysregulated by the treatment by both platforms were very similar and related to chemical carcinogenesis. Tumorigenesis and apoptosis were identified as the top functional pathways for both platforms. These results indicate that while the alteration of gene expression determined by NGS is comparable to that by microarray in terms of the biological functional analysis, NGS is more sensitive than microarray for detecting DEGs.

PS 127 THE ARYL HYDROCARBON RECEPTOR PROTEIN INTERACTION NETWORK (AHR-PIN).

J. J. LaPres[1, 2], D. M. Tappenden[1, 2], L. Yang[3] and R. S. Thomas[3]. [1]*Biochemistry, Michigan State University, East Lansing, MI,* [2]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [3]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

The aryl hydrocarbon receptor (AHR), a ligand-activated PAS super-family transcription factor member, is responsible for controlling most, if not all, of the toxic effects of environmental pollutants, such as polyaromatic hydrocarbons (PAHs).

PAHs, such as dioxins, are prevalent contaminates that are produced by both natural and man-made activities. 2,3,7,8 tetrachloro-dibenzo-p-dioxin (TCDD), is the prototypical dioxin and a group I carcinogen. TCDD-induced toxicity has species and tissue specificity, indicating the complex nature of AHR-mediated signaling and biology. To date, a complete understanding of the AHR's role on cellular processes and how these are influenced in TCDD exposure remain incomplete. To explore the potential influences of protein-protein interactions in AHR-mediated signaling and toxicity, tandem affinity purification (TAP) and mass spectrometry strategy was utilized to isolate and identify proteins that co-purify with the AHR. This methodology was validated by the identification of the known protein interactions between the AHR, Hsp90, and ARA9 and the ligand dependant association with ARNT. Furthermore, using this technology, novel interactions for the AHR have been indentified that connect the receptor to cellular energetics, cell cycle regulation, cell death, mitochondrial function, and development. Here we present the identification of the AHR-PIN and characterization of TCDD-induced changes in the network.

PS 128 SEQUENCE TAGGING REVEALS UNEXPECTED MODIFICATIONS IN TOXICOPROTEOMICS.

S. Dasari[1], M. C. Chambers[1], D. C. Liebler[2], F. P. Guengerich[2] and D. L. Tabb[1,2]. [1]*Biomedical Informatics, School of Medicine, Vanderbilt University, Nashville, TN and* [2]*Biochemistry, School of Medicine, Vanderbilt University, Nashville, TN.*

Rationale: Improved bioinformatics for detecting unexpected chemical and post-translational modifications in toxicoproteomic datasets.Toxicoproteomics produces tandem mass spectra (MS/MS) that contain all the information needed to identify modified peptides. Identifying these variant peptides, however, is intractable with traditional database search engines. We have developed new software (TagRecon) that leverages inferred sequence tags for detecting known and unknown modifications present in toxicoproteomic datasets. TagRecon is integrated into a bioinformatics pipeline containing a protein assembler and a user-friendly modification results reviewer. The pipeline produces HTML reports of proteins, peptides, and modifications. Processing a diverse collection of toxicoproteomic datasets with the pipeline revealed a surprisingly wide palette of unanticipated modifications, none of which were apparent via standard database search. We tested TagRecon with a total of 1.5 million MS/MS produced from three complex toxicoproteomic experiments: liver lysates of rats administered with five potentially hepatotoxic drugs; THP1 cell lines treated with alkynylhydroxynonenal (alk-HNE); DNA/histone complexes treated with 1,2-dibromoethane (EDB) and diepoxybutane (BDE). TagRecon improved peptide identification rates by 50%-70% vs. the state-of-the-art InsPecT software. TagRecon revealed 25 proteins in rat liver samples (including microsomal glutathione s-transferase 1, a known drug metabolite) that were targets of drug-induced oxidative stress. In the DNA/histones sample, we detected unintended cross-reactions between EDB and dimethylated histone lysines. Proteins in the THP1 cell lines dataset contained unanticipated alk-HNE adducts. These experiments demonstrate that toxicoproteomic datasets contain important additional information that can be revealed through improved bioinformatics. The complete set of tools is freely available from http://fenchurch.mc.vanderbilt.edu.

PS 129 PROTEOMIC PROFILING OF DYNAMIC CELLULAR RESPONSES TO SILICA NANOPARTICLES USING STABLE ISOTOPE AMINO ACID LABELING.

B. Thrall[1], B. Webb-Robertson[2], K. Burnham[3], M. H. Littke[1], N. J. Karin[3], J. Jacobs[3] and K. M. Waters[2]. [1]*Cell Biology & Biochemistry, PNNL, Richland, WA,* [2]*Computational Biology & Bioinformatics, PNNL, Richland, WA and* [3]*Biological Separations & Mass Spectrometry Groups, Pacific Northwest National Laboratory, Richland, WA.*

In addition to transcriptional events, selective alterations in protein synthesis, stability and degradation underlie many key cellular processes linked to pathological outcomes. Motivated by the hypothesis that cellular responses to engineered nanoparticles (ENPs) may be manifested by dynamic post-transcriptional events, we conducted a proteomic analysis of the effects of SiO2 ENP exposure on mouse lung epithelial cells. Our strategy involved mass spectrometry analysis of the metabolic incorporation of a stable isotope labeled amino acid (+8 Da lysine) into proteins over time to quantify the impacts of ENP treatment on levels of newly translated proteins in mouse C10 cells during exposure to a threshold cytotoxic dose of 50 nm SiO2 ENPs for 1, 3, 5, 8 or 14 hrs. Among 457 proteins (777 peptides) identified with sufficient data across time point for statistical analysis, over 200 proteins showed significantly different ($p<0.05$) lysine incorporation compared to untreated controls. Proteins whose synthesis increased in response to ENP were found, such as the redox stress marker heme oxygenase, which was verified by RT-PCR to be transcriptionally controlled. However, clustering and principal components analysis showed the predominant effect of ENP exposure was a suppression/delay in synthesis of select groups of proteins, including proteins involved in translational regulation. Genomic and RT-PCR data showed these responses are accompanied by early transcriptional induction of ATF3, ATF4 and ATF6 genes, transcription factors involved in regulating mammalian unfolded protein responses and feedback inhibition of inflammatory signaling cascades. The integrated data suggest the cell response to SiO2 ENPs mimics an adaptive ER-stress response, for which ATF family gene products may serve as early biomarkers.

PS 130 ENVIRONMENTAL CHEMICALS AND HUMAN HEALTH: UNCOVERING THE CONNECTIONS WITH CTD.

C. Mattingly, C. G. Murphy, C. A. Saraceni-Richards, S. Mockus, M. C. Rosenstein, T. C. Wiegers, B. King and A. P. Davis. *Bioinformatics, MDI Biological Laboratory, Salisbury Cove, ME.*

The Comparative Toxicogenomics Database (CTD) is a freely available resource (http://ctd.mdibl.org), funded by the National Institute of Environmental Health Sciences, which provides unique data and analysis tools to promote understanding about the effects of the environment on human health. CTD Biocurators manually capture chemical-gene/protein interactions and chemical- and gene-disease relationships from the peer-reviewed biomedical literature. These data are integrated with select public data sets (e.g., Gene Ontology annotations and pathways from KEGG and Reactome) to support development of novel hypotheses about chemical actions and etiologies of environmentally influenced diseases. Currently, CTD contains approximately 250,000 chemical-gene/protein interactions, 12,000 gene-disease and 9,000 chemical-disease relationships – relationships inferred from these data exceed 1.2 million. To facilitate navigation and interpretation of these data, we provide an increasing range of analysis tools: a) statistical measures are now calculated that rank inferred data relationships to help prioritize hypothesis development; b) enriched Gene Ontology terms are calculated for chemicals to enhance understanding about their biological effects; c) "Batch Query" allows users to retrieve any type of data in CTD (e.g., molecular interactions, enriched GO and KEGG annotations) associated with a list of their chemicals, genes, or diseases of interest; d) "VennViewer" and "MyGeneVenn" generate diagrams to compare specified CTD data associated with up to three chemicals, diseases, or genes (including a user's own gene list) of interest; and e) "GeneComps" and "ChemComps" identify similar genes or chemicals based upon shared toxicogenomic profiles. Collectively, CTD's wealth of information, data integration strategy and unique analysis tools make it an unparalleled and flexible resource for developing hypotheses about the mechanisms underlying environmental diseases.

PS 131 GENERATING A GENE NETWORKS ASSOCIATED WITH KOLA ACUMINATA-BIZZY NUT INDUCE APOPTOSIS.

R. Ledet and W. G. Gray. *Chemistry, Southern University A&M College, Baton Rouge, LA.*

Kola acuminata, also known as Bizzy Nut or Kola Nut, is a natural product that contains bioactive chemicals that possess hormonal properties. Work from our laboratory has shown that Bizzy may be chemopreventative toward both breast and prostate tumors. Tumor growth is influenced by several factors, which result in differential gene expression and function. The gene network that is involved in the development of tumors from specific environmental factors is largely unresolved. Furthermore, the gene networks associated with Bizzy's chemopreventative and antitumor activity, which affect prostate and breast cancer development, has yet to be explored. Given that Bizzy contains putative chemopreventative and anticancer properties, we hypothesized that Bizzy will modulate the expression level of genes that are involved in tumor carcinogenesis pathways. Therefore, the objective of this study is to generate a Bizzy specific gene network map by identifying, analyzing, and characterizing Bizzy-responsive genes. As an initial step in accomplishing our objective, we have generated a putative Bizzy gene network with associated biochemical pathways. Regulation of these genes by Bizzy has been studied using PCR and western blot analysis. We have expanded gene the network by generating a Bizzy-responsive prostate-specific regulatory network using a prostate-specific subtraction cDNA library. The plasmid DNA identity will be confirmed by electrophoresis and PCR. The cDNA library has been arrayed into several 384 gene clusters and their size, location, DNA sequence, level of expression, and function will be determined. Two Bizzy-responsive cDNA (pDP1.3 and pAR) have been isolated and sequenced and their position in the network has been determined. Our initial Bizzy network demonstrates a correlation between genes involved in cell proliferation and apoptosis in the presence of Bizzy. The availability of this resource (Bizzy-specific gene map) will allow us to make conclusions concerning the efficacy of Bizzy in prostate cancer prevention.

PS 132 VIRTUAL SCREENING OF DIFFERENTIAL PROTEIN TARGETS FOR TROVAFLOXACIN AND LEVOFLOXACIN.

J. Olivero-Verbel, C. Ortega-Zuñiga and M. Cabarcas-Montalvo. *Environmental and Computational Chemistry Group, University of Cartagena, Cartagena, Bolivar, Colombia.*

Trovafloxacin (TVX) and levofloxacin (LVX) are fluoroquinolones with proven in vitro and in vivo efficacies as antimicrobial agents. Both exert their antibacterial effects by inhibiting bacterial DNA synthesis. TVX use was restricted in 1999 due to its link with idiosyncratic hepatotoxicity, property that has not been observed with LVX. In silico virtual screening was used to identify theoretical protein targets with distinct affinities for both compounds, aiming to explain some of their reported toxicological differences. Protein-ligand docking approaches were performed to estimate the binding affinity of these molecules in several proteins known to be involved in their biochemical effects. Protein structures were obtained from Protein Data Bank and prepared with SYBYL 8.1 and MGL Tools (1.5.0). Three-dimensional structures of antibiotics were optimized by quantum chemical protocols, and then docked to proteins using AutoDock Vina program. Human adenosine A2A receptor presented a high binding affinity score for TVX (-11.2 Kcal/mol), whereas it was moderate for LVX (-8.4 Kcal/mol). Similarly, matrix metalloproteinase 1, 8, 10, 13 and 16 showed discriminative binding affinities for TVX compared to LVX that varied between 2-4 units. Ligand-protein interactions on the binding site of these proteins revealed that the antibiotics docked differently on the target despite of being structurally similar. In addition, the number of interactions found for TVX was greater compared with LVX, being mostly hydrophobic in nature. A2A receptor and most of the matrix metalloproteinases reported here are key mediators in immune/inflammatory diseases of various organs, including the liver. Accordingly, these results suggest that, at least hypothetically, TVX and LVX target some proteins with differential binding affinities, probably leading to the differences in the toxicity profile observed for these chemicals. Colciencias-University of Cartagena (110745921616).

PS 133 THE MOUSE GENOME INFORMATICS DATABASE: INTEGRATING INFORMATION TO FACILITATE DISCOVERY.

S. M. Bello, C. L. Smith, H. Dene, D. L. Burkart, L. L. Washburn, M. Tomczuk, A. Anagnostopoulos, B. Richards-Smith, H. Onda, R. P. Babiuk, M. Knowlton, J. Xu, J. A. Bubier and J. T. Eppig. *Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME.*

The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org) is the free international resource for the genetics, genomics, and biology of the laboratory mouse. Many different types of biological data (i.e. mouse mutant phenotypes, relationships of genes and gene products to functions, developmental gene expression, genome location) from a variety of sources are integrated within MGI. This integration allows users to search, compare, and analyze data that are not effectively retrieved through text searches. To access these integrated resources, users may either use the quick search tool or database query forms. The quick search tool allows users to search for specific genes, alleles, diseases, or keywords. For example one can search for "angiogenesis" to find all genes, alleles and vocabulary terms that are associated with this process. The gene results may then be used to search for additional information about the list of genes, including Gene Ontology, Mammalian Phenotype or expression annotations. Query forms allow users to combine multiple criteria to take advantage of the integration of data within MGI or to search for information about multiple genes at once. For example using the batch query one can ask "What are the phenotypes produced by mutations in genes that are induced by ribotoxic stress?" Results from the query forms may be viewed on the web, exported directly to an excel spreadsheet, or sent in tab delimited format to the MGI ftp site and downloaded. New features added in the past year include the addition of feature types for genes (such as non-coding RNA or heritable phenotypic marker), the incorporation of Eurexpress expression data, and inclusion of all KOMP and EUCOMM generated alleles. The Mouse Genome Database (MGD) at MGI is supported by NIH/NHGRI grant HG0000330.

PS 134 FINDING CLUSTERS OF CHEMICALS AFFECTING SUB-NETWORKS OF THE PROTEIN INTERACTOME.

R. Thomas[1], I. Gerlovina[1], A. Hubbard[1], M. North[2] and C. Vulpe[2]. [1]*Biostatistics, University of California, Berkeley, Berkeley, CA and* [2]*Nutritional Science and Toxicology, University of California, Berkeley, Berkeley, CA.*

Mechanistic toxicological researchers are typically interested in molecular mechanisms through which an exposure to a chemical leads to a toxicological end point. One mechanism of generating hypotheses regarding the toxicological end points for these compounds would be to compare the signatures of molecular measurements of the less understood compounds with the similar output for the relatively well understood chemicals. The question then becomes how to effectively generate these signatures. Approaches could be based on signatures generated from arbitrary sets of genes or well-defined sets of genes that are known to participate in known biological processes. A novel statistically-based network method is proposed that uses a system-wide analysis of the protein-protein interaction network in order to identify sub-networks of proteins modulated by a group of chemicals. Sub-networks significantly enriched by single chemicals, pairs of chemicals and further combinations of chemicals are identified. The novelty of the methodology is in its direct use of the network characteristics (like the number of interactions that each protein is involved in, the number of interactions separating two proteins) of the actual protein-protein interaction network of the organism. The methodology is applied to data from yeast knockout growth assays for a group of eight Superfund chemicals – arsenic, MMA3, lead, zinc, cadmium, catechol, benzenetriol and hydroquinone. The yeast protein interaction network from the STRING database is used. Networks significantly affected by arsenic and its metabolite MMA3 and networks modulated by the metabolites of benzene are identified. Sub-networks involving vesicular transport proteins seem to be significantly enriched for all these chemicals suggesting the possibility of defining this network as a common toxicity pathway.

PS 135 A BIOINFORMATICS-BASED PIPELINE FOR BIOMARKER DISCOVERY UTILIZING BOTH *IN VITRO* AND *IN VIVO* DATA.

R. C. Grafström[1, 2], R. Ceder[1], C. A. Staab[3], J. Höög[3], H. Jörnvall[3], M. Nees[2], B. Fadeel[1] and K. Roberg[4]. [1]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden,* [2]*Medical Biotechnology, VTT Technical Research Centre of Finland, Turku, Finland,* [3]*Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden and* [4]*Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden.*

We constructed an omics data-driven pipeline for biomarker discovery considering that it should be widely applicable to biomedical research. As proof of concept, proteomics (2D-PAGE with subsequent MALDI-TOF or LC-MS/MS) transcriptomics (microarray) and multiple sequential bioinformatics tools were applied to define the genotype of the LK0412 tongue squamous cell carcinoma line relative to the normal state. A set of 27 abundant differently expressed proteins implied alterations related to cancer, cellular movement/assembly/organization, cell death, protein metabolism and energy pathways (including the involvement of 10 gene ontology (GO) categories, 119 transcripts and 11 key regulator genes located centrally in molecular networks). The proteins, transcripts, and eleven-gene profile, respectively, separated a training set of public microarray data from normal tissue and tongue tumor specimens with an accuracy of 80-86%. Statistical signature evaluation improved the discrimination of normal and tumor tissue such that ultimately an eight-gene signature averaged an accuracy of 90% in three independent oral/head and neck cancer data. Differently, six non-oral tumor sets (blood, brain, breast, kidney, lung, sarcoma) where predicted to considerably lower levels. Some signature genes were previously coupled to tongue/oral carcinoma as indicated from verifications relative to transcriptomics, proteomics, saliva and plasma protein databases. Predictive limited-number gene signatures generated under the pipeline protocol are potentially widely applicable to the field of computational toxicology.

PS 136 CHARACTERIZATION OF A QSAR MODEL FOR PHOSPHOLIPIDOSIS USING A HIERARCHICAL SCAFFOLD TREE OF SIGNIFICANT MOLECULAR FEATURES.

N. L. Kruhlak[1], K. Cross[2] and A. M. Orogo[1, 3]. [1]*U.S. FDA/CDER/OTR/DAPR, Silver Spring, MD,* [2]*Leadscope Inc., Columbus, OH and* [3]*GlobalNet Services Inc., Rockville, MD.* Sponsor: R. Benz.

Drug-induced phospholipidosis (PL) continues to be a safety concern for pharmaceutical companies and regulatory agencies, which has prompted the FDA/CDER Phospholipidosis Working Group to establish a database of PL findings from the published literature, FDA archives, and pharmaceutical industry donors. This database was recently enhanced to include PL data linked to chemical structures for an additional 200 compounds to yield a total of 743 compounds (385 positive and 358 negative) for the purposes of quantitative structure-activity relationship (QSAR) modeling. Several commercially available software programs were investigated for their ability to generate models to predict PL induction. One platform (Leadscope's Predictive Data Miner) gave performance statistics of 78.0% specificity, 79.0% sensitivity, and 78.5% concordance in cross-validation experiments of models constructed using partial logistic regression analysis and Leadscope's library of molecular fingerprints. The final optimized model was based on 283 molecular features and properties and supported the widely acknowledged correlation between cationic amphiphilic drugs and PL induction by identifying basic nitrogen-

containing substituents and/or hydrophobic ring systems as being significantly correlated with PL activity. Model features and scaffolds were organized using a hierarchical scaffold tree to illustrate their relatedness and their ability to predict PL independently. Closer examination of subsets of related scaffolds show the effects of various molecular substitution patterns on the correlation with PL activity, as well as underscore the need to be judicious in selecting molecular features that best predict the training set without overly compromising the model's domain of applicability by defining the local structure environment too narrowly.

PS 137 *IN SILICO* PREDICTIVE MODEL FOR DRUG-INDUCED PHOSPHOLIPIDOSIS USING BIOEPISTEME SOFTWARE.

S. S. Choi, L. G. Valerio and N. Sadrieh. *U.S. FDA, Silver Spring, MD.*

Drug-induced Phospholipidosis (DIPL) is a recognized finding in pharmaceutical drug development. DIPL is characterized by accumulation of drugs and phospholipids in lysosomes. Pathologically, DIPL manifests foamy macrophages or cytoplasmic vacuoles in various tissues of both animals and humans. These pathologic findings can be confirmed by the appearance of lamellar inclusion bodies by electron microscopy. CADs (cationic amphiphilic drugs) are known to induce PL and share common structural features of containing hydrophobic ring structure and hydrophilic amine portion. This has led FDA to investigate the structural similarities and differences for active and inactive compounds. FDA has been developing a PL database that currently contains over 700 PL positive and negative drugs. Using this database and various computational programs, FDA has developed predictive QSAR models for DIPL. In this study, new In silico models were generated and validated using BioEpisteme, a toxicity screening and QSAR model development program, developed by the Prous Institute for Biomedical Research. BioEpisteme facilitates creation and application of In silico toxicity predictions based on molecular descriptors. In addition, it provides predictions on mechanism of action (MOA) (mechanism of action) for 432 different pharmacological targets. Preliminary performance statistics of a new DIPL QSAR model using a genetic algorithm of BioEpisteme show 87% specificity, 73% sensitivity, and 82% accuracy. These results were compared with other available FDA QSAR models. In addition, the MOA model was investigated to predict possible pharmaceutical MOA targets of the DIPL data set. These highly performing In silico predictive models will help to predict DIPL early in drug development and will subsequently serve to support regulatory decisions.

PS 138 PREDICTION OF DRUG-INDUCED LIVER INJURY IN HUMANS WITH TOXICOGENOMICS.

M. Zhang, Q. Shi, M. Chen and W. Tong. *NCTR, U.S. FDA, Jefferson, AR.*

Drug induced liver injury (DILI) is a leading cause of liver failure and the withdrawal of drugs from the market. However, the mechanism of DILI is still not well understood, and the prediction of the DILI potential for specific drugs remains a challenge. Over the past few years, large-scale microarray experiments testing DILI have been performed on animal models and have provided a good opportunity to understand DILI on the gene expression level. Combining our recently developed liver toxicity knowledge base- benchmark database (LTKB-DB), which groups drugs by DILI potential based on their drug labels, we used the microarray data from a previous toxicogenomic study to distinguish the DILI potentials of drugs by analyzing gene expression profiles of rats treated by those drugs. We focused on eighteen drugs with different DILI potentials and achieved a classification accuracy of over 80% using the leave one compound out cross validation process, which not only provided in silico evidence for drug categories in LTKB-DB, but also suggested the feasibility of predicting drug induced liver injury using microarray expression data. Additionally, the effects of the ALT/TBL level in the treated samples on the DILI potentials of the drugs were also discussed.

PS 139 PREDICTIVE VALUE OF CHEMICAL AND TOXICOGENOMIC DESCRIPTORS FOR DRUG-INDUCED HEPATOTOXICITY.

Y. Low[1, 5], T. Uehara[1, 2], Y. Minowa[2], H. Yamada[2], Y. Ohno[3], T. Urushidani[2, 4], A. Sedykh[5], D. Fourches[5], H. Zhu[5], I. Rusyn[1] and A. Tropsha[5]. *[1]Environmental Sciences & Engineering, University of North Carolina, Chapel Hill, NC, [2]Toxicogenomics Informatics Project, National Institute of Biomedical Innovation, Osaka, Japan, [3]National Institute of Health Sciences, Tokyo, Japan, [4]Doshisha Women's College of Liberal Arts, Kyoto, Japan and [5]Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC.*

Quantitative Structure-Activity Relationship (QSAR) modeling and toxicogenomics have been extensively explored independently as predictive toxicology tools. In this study, we employed a rigorous QSAR modeling workflow to predict the hepatotoxicity for 127 well-known drugs in the Toxicogenomics Informatics Project using both approaches. Predictor variables included chemical descriptors as well as biological descriptors represented by the short-term gene expression profile for rat liver (24h after dosing). The prediction target, hepatotoxicity, was defined by histopathology and serum chemistry results after 28 days of treatment. We first developed conventional QSAR models using a comprehensive set of chemical descriptors and several classification methods. Using only chemical descriptors, external predictivity expressed as Correct Classification Rate (CCR) and evaluated by 5-fold external cross-validation was as high as 59%. In parallel, models were built using only gene expression data. Optimal models comprising of 85 genes had CCR of 76%. Finally, hybrid models combining both chemical descriptors and gene expression data were also developed but their predictivity was under 76%. Although the accuracy of the QSAR models involving only chemical descriptors was limited due to the high chemical diversity of the small dataset, using both chemical and biological descriptors enriched the interpretation of the modeling results: the 85 predictor genes were mechanistically relevant, involving hepatotoxicity-related pathways, and structural alerts were identified among hepatotoxicants. This study shows that concurrent exploration of chemical features and early treatment-induced changes in gene expression affords predictive and interpretable models of subacute hepatotoxicity.

PS 140 CLASSIFICATION OF HUMAN CYTOCHROME 3A4 LIGANDS BY MEANS OF MOLECULAR DOCKING.

Y. Tie[1], B. T. McPhail[1], H. Hong[2], R. D. Beger[2], B. A. Fowler[1] and E. Demchuk[1]. *[1]Division of Toxicology & Environmental Medicine, ATSDR/CDC, Atlanta, GA and [2]Division of Systems Biology, NCTR/U.S. FDA, Jefferson, AR.*

An interagency collaboration of ATSDR/CDC and NCTR/FDA was developed to model adverse drug-drug interactions mediated by CYP3A4 and 2D6. The modeling of CYP3A4 was carried out using several computational methods, including molecular docking. Molecular docking is a technique frequently used to estimate the binding interactions between ligands and a protein. Initially, 121 drugs were classified as strong and weak binders of CYP3A4. Docking studies were then performed by eHiTS, FRED, Glide, and SYBYL. Docking scores were used to develop multiple logistic regression (LR) models. The model with the least number of parameters (specificity of 100%, sensitivity of 42.4%, and overall accuracy of 84.3% at a LR probability cutoff of 0.5) was selected for final modeling and cross-validation (CV). A 10-fold CV was used. The average accuracy at the CV stage was 83.8% and 79.3% for the training and CV sets, respectively. These results were compared to those of two other models: SDAR (spectrum-data-activity relationship) and a DF-SAR (decision forest structure-activity relationship). Although the accuracy of classification was comparable among the modeling methods, the number of parameters, and consequently the robustness of the models, was different. While only 7 parameters were used in the LR docking, 27 descriptors were used in SDAR, and even a greater number in the DF-SAR: 5 trees ranging from 5 to 17 descriptors. All three models were used to predict which ligands in a test set of 120 were strong or weak binders. In summary, molecular docking provides a unique way of CYP3A4 ligand classification, which is complementary to traditional S(D)AR modeling. While S(D)AR techniques are focused at intra-molecular properties, molecular docking takes account inter-molecular interactions. The three modeling methods combined provide an *a priori* assessment of hepatic drug-drug interactions and could serve as an adjunct to the conventional FDA drug approval process and to the public health review process of chemical safety by ATSDR.

PS 141 BUILDING STRUCTURE FEATURE-BASED MODELS FOR PREDICTING ISOFORM-SPECIFIC HUMAN CYTOCHROME P450 (HCYP 3A4, 2D6 AND 2C9) INHIBITION ASSAY RESULTS IN TOXCAST.

P. Volarath[1], B. Bienfait[2], J. Gasteiger[2], K. Houck[1] and A. Richard[1]. *[1]NCCT, U.S. EPA, Research Triangle Park, NC and [2]Molecular Networks GmbH, Erlangen, Germany.*

EPA's ToxCast project is using high-throughput screening (HTS) to profile and prioritize chemicals for further testing. ToxCast Phase I evaluated 309 unique chemicals, the majority pesticide actives, in over 500 HTS assays. These included 3 human cytochrome P450 (hCYP3A4, hCYP2D6, hCYP2C9) inhibition assays that are routinely used to evaluate drug candidates for potential drug-drug interactions. isoCYP is a structure-activity relationship (SAR) prediction model built on a training set of hCYP isoform-specific metabolism data that uses a decision-tree approach to predict which of the these 3 CYP isoforms is preferentially involved in drug metabolism. The published isoCYP models were derived from a training set of 146 drugs for which the particular single metabolizing CYP isoform is known in each case (Terfloth et al., 2007, J.Chem. Inf. Model., 47:1688-1701). ToxCast results for the 3 hCYP inhibition assays differ significantly from the isoCYP model

data, both in terms of the chemical space (industrial/agro chemicals vs. drugs) and in terms of the hCYP activity profiles, which for ToxCast include a large proportion (80%) of chemicals inactive in all 3 hCYP inhibition assays. The ToxCast inhibition assay results and the isoCYP metabolic activity data together afford the opportunity to explore SAR associations for a significantly expanded chemical space, using both features and chemical property descriptors to discern requirements for qualitative hCYP activity, as well as hCYP isoform specificity. ToxCast Phase II is screening an additional 700 chemicals, including expanded sets of drugs and agro/industrial chemicals, in the same 3 hCYP inhibition assays, providing an ideal opportunity to prospectively evaluate the performance of the new models, as well as to iteratively improve upon these models.
This abstract does not necessarily reflect U.S. EPA policy.

PS 142 EFFECTS OF GENETIC VARIATION IN ENZYME CYTOCHROME P450 2D6 ON XENOBIOTIC METABOLISM THROUGH *IN SILICO* MOLECULAR DOCKING MODELS.

C. B. Tolson[1, 2], Y. Tie[1] and E. Demchuk[1]. [1]*Division of Toxicology & Environmental Medicine, ATSDR/CDC, Atlanta, GA and* [2]*Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA.* Sponsor: B. Fowler.

Most drug metabolism performed in the liver is a result of enzymatic activity of the cytochrome P450 family of enzymes, especially cytochrome P450 3A4 and 2D6. Genetic variation is a large source of enzyme activity variability. This project determined whether methods of computational molecular docking can be used to detect the effects of genetic variation on substrate binding. The project is part of a large joint program of ATSDR/CDC and NCTR/FDA aimed at modeling liver toxicity. The project concentrates on enzymatic activity in CYP2D6 variants with the 120 residue substitution (CYP2D6*53 and CYP2D6*49). Computational molecular docking modeling using FRED, Induced-Fit, and GLIDE was applied. The *in silico* docking models analyzed the interactions between individual ligands and the active sites of the CYP2D6 enzyme variants. The results suggested that computational molecular docking models could be used as accurate predictors in pre-clinical drug testing, but may have limited sensitivity to genetic variations beyond the active site. The FRED docking model best suited for the CYP2D6 enzyme-substrate system. Since data on human cytochrome P450 metabolism and associated genetic susceptibility have been obtained almost exclusively in clinical drug testing, better understanding of the molecular interactions between xenobiotics and the cytochrome P450 family will aid in the development of better predictive computational models to be used during pre-clinical drug development. Once a robust human model is developed, it can be applied in other areas, including chemical and biological defense and environmental health problems, such as estimation of metabolic parameters in physiologically-based pharmacokinetic modeling (PBPK) or more specific recommendations for toxic substance dosages based on genetic variants within a particular population.

PS 143 INCORPORATION OF TOXCAST *IN VITRO* ASSAY DATA AND RELEVANT TOXICITY PATHWAY INFORMATION IMPROVES THE EXTERNAL PREDICTION ACCURACY OF QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) MODELS OF CHEMICAL HEPATOTOXICITY.

H. Zhu, L. Zhang, J. Staab, A. Sedykh, H. Tang, S. Gomez, I. Rusyn and A. Tropsha. *University of North Carolina at Chapel Hill, Chapel Hill, NC.*

The US EPA ToxCast program provides data for 610 in vitro assays, in which 320 substances with known in vivo toxicity measured in 76 assays were tested. We have explored this complex data with novel computational approaches aimed to link chemical structures, in vitro responses, pathway information, and in vivo toxicity effects. We have used data from 22 ToxCast assays linked to Peroxisome Proliferator-Activated Receptor (PPAR) pathway to build biologically inferred QSAR models of six chronic animal hepatotoxicity endpoints. The datasets included 228 and 235 compounds for mouse and rat hepatotoxicity subsets, respectively, and both conventional QSAR models and those using the following novel hierarchical QSAR modeling workflow were built. First, all chemicals were partitioned into two classes based on whether a compound was active (Class I) or inactive (Class II) in any of the PPAR-relevant assays; we found that there were 100 Class I compounds and 128 Class II compounds for the mouse data and 106 Class I compounds and 129 Class II compounds for the rat data. Second, classification hepatotoxicity models for each endpoint were developed using both random forest and MOE binary QSAR approaches for Class I and II compounds, independently. The prediction for external compounds is realized by initially placing them into Class I or II based on in vitro responses and then using class-specific QSAR models for in vivo toxicity prediction. The external prediction accuracy of models built with this two-step workflow was in the range of 57-76% for all six hepatotoxicity endpoints, while that achieved by conventional QSAR models was only 50-65% for the same external set. We conclude that methods discussed herein offer novel ways of exploiting biological screening data to improve the accuracy of predictive toxicology models.

PS 144 COMPUTATIONAL DOCKING OF THE ISOMERS OF NONYLPHENOL TO THE LIGAND BINDING DOMAIN OF THE ESTROGEN RECEPTOR.

J. Rabinowitz and S. Little. *NCCT/ORD/Environmental Protection Agency, Research Triangle Park, NC.*

Nonylphenols are environmentally persistent endocrine disrupting chemicals. They exist in the environment as complex mixtures containing many nonylphenol isomers. Environmental mixtures of nonylphenols, along with a few single isomers have been tested for their capacity to interact with the estrogen receptor (ER) and have been shown to be weakly estrogenic. The few individual isomers tested have only in rare examples been shown to be more estrogenic than environmental mixtures of undetermined isomers. The capacity of a molecule to bind to ER depends on the three dimensional structure of the potential ligand. There are 211 geometric isomers and 551 stereo isomers of nonylphenol. The potential for each isomer to bind to ER will depend on the energetics the interaction. Computational molecular docking has been used to examine the capacity of each nonylphenol isomer to bind to ER. Computational targets have been created by removing the ligand atoms from crystal structures of ER-ligand complexes. The isomers of nonylphenol have been docked sucessfully into these targets. The results indicate that the potential for binding will be greatest when the molecular structure maximizes both the strength of the hydrogen bonds between the receptor and the phenolic hydroxyl group, and the steric interaction between the alkyl group and the hydrophobic core of the receptor. For some isomers the formation of the necessary hydrogen bonds forces the ligand-receptor complex into a structure with less favorable steric interactions. For example, branching at the alpha position decreases the interaction while branching in the beta and gamma positions often increases the interaction. Analysis of these considerations can provide insight for the design and manufacture of nonylphenol (and other alkylphenol) mixtures that minimize their potential estrogenicity. [This abstract does not necessarily reflect U.S. EPA policy.]

PS 145 ANALYSIS OF RELATIVE ROSIGLITAZONE, PIOGLITAZONE, AND EPIRUBICIN CARDIOTOXICITY IN RATS USING HISTORICAL MICROARRAY DATA.

H. Zhou and T. J. Colatsky. *CDER/OPS/OTR/DAPR, U.S. FDA, Silver Spring, MD.*

Concerns have been raised about increased cardiovascular risk in patients treated with rosiglitazone (ROSI), but ROSI's cardiovascular safety profile remains an open question because of conflicting data on the existence and magnitude of the risk. We evaluated rat heart microarray data extracted from the Iconix DrugMatrix (IDM) database for ROSI (5d 1800 mg/kg/day; IDM high dose) and pioglitazone (PIO; 5d 1500 mg/kg/day; IDM high dose), and compared the results to similar data obtained for epirubicin (EPI; 5d 2.7mg/kg/day; IDM high dose), a drug with well characterized cardiotoxicity. ROSI treatment induced 1002 differentially expressed genes (DEGs) in rat heart compared to 360 DEGs for PIO and 986 DEGs for EPI. Further analysis using Ingenuity Pathways Analysis revealed that ROSI and EPI induced more than twice the number of cardiotoxicity-related DEGs than PIO treatment but fewer than EPI (EPI:ROSI:PIO = 100:88:40). All 3 drugs were associated with NRF-2-mediated Oxidative Stress Response (EPI>ROSI>PIO), while ROSI and EPI both impacted Oxidative Stress and VDR/RXR activation. In addition, ROSI treatments altered 20 cardiac hypertrophy related molecules while PIO altered only 6. These results could be correlated with the increase in heart weight produced by ROSI and PIO in these studies. Among the cardiotoxicity related molecules demonstrating altered expression, ROSI and PIO shared 17/111 common DEGs, ROSI and EPI shared 22/166 common DEGs, and PIO and EPI shared 13/127 common DEGs while all the three shared only 6 common DEGs: CCNA2, CD36, FOS, HOPX, NCALD and PRKCH. In conclusion, these data suggest ROSI may be more cardiotoxic than PIO in rats, but the large number of unique cardiotoxicity related DEGs suggested multiple pathways exist for the cardiovascular effects of ROSI, PIO and EPI treatment. However, the relevance of these analyses to the more complex clinical situation still needs to be assessed.

PS 146 COMPUTATIONAL MODELING FOR QT PROLONGATION: A DRUG CARDIOVASCULAR SAFETY ENDPOINT OF PARAMOUNT IMPORTANCE.

L. G. Valerio[1], J. Prous Blancafort[2], A. Valencia[2] and X. Mensa[2]. [1]*CDER/OPS, U.S. FDA, Silver Spring, MD and* [2]*Prous Institute for Biomedical Research, Barcelona, Spain.* Sponsor: N. Sadrieh.

Because of the potentially catastrophic nature of unanticipated "off-target" drug-related cardiovascular effects, adequate assessment of cardiac safety is of paramount importance. Torsades de pointes (TdP), which is a potentially lethal cardiac arrhythmia,,has been observed with a number of drugs, after they were approved and marketed. Prediction of such a lethal adverse event, prior to approval and marketing of the product, would have allowed for a more thorough regulatory assessment of clinical trial data, and potentially could have halted the clinical trials and terminated the drug's development. QT interval prolongation is a precursor to TdP, and thus predicting this effect would certainly allow for better clinical development of candidate drug products. The measurement of QTc interval effects has been adopted into the guidance set by the FDA and international health authorities to assess pro-arrhythmic potential for new drugs. In recognizing this public health concern and the goal of developing better predictive methods to minimize pro-arrhythmic liability, our group has deployed a computational modeling approach based on new high quality human clinical trial data. The clinical data on QT prolongation during clinical cardiovascular studies will support the development of innovative qualitative and quantitative structure-activity models that can help predict QT prolongation and TdP events, based on chemical structure. This present effort is the first that we know of, where human clinical trial data will be used to construct predictive computational toxicology models to help screen drugs for QT prolongation and subsequently TdP, and will serve as a decision support tool for risk assessment of new drugs.

PS 147 TRANSLATABILITY OF DRUG INDUCED CARDIOTOXICITY MOLECULAR MECHANISMS FROM *IN VIVO* TO *IN VITRO.*

A. Enayetallah[1], D. Puppala[1], D. Ziemek[2], N. Greene[1], Y. Will[1] and M. Pletcher[1]. [1]*Compound Safety Prediction-PGRD, Pfizer Inc., Groton, CT and* [2]*Computational Sciences CoE, Pfizer Inc., Cambridge, MA.*

One of the major hurdles in developing in vitro assays to assess cardiac safety of pharmaceutical compounds is the lack of understanding of how reflective the cell-based in vitro models are of in vivo toxicity. The goal of this study is to identify drug-induced cardiotoxicity mechanisms that are translatable from in vivo to in vitro. In vivo gene expression data collected from rat hearts after treatment with known cardiotoxic compounds were obtained from the Iconix DrugMatrix database for 10 different compounds. To generate comparable in vitro gene expression datasets, two cell lines of cardiomyocyte origin, H9C2 and 1ry rat cardiomyocytes were treated with the same 10 compounds from the in vivo studies at their cell toxicity IC20 and IC50 concentrations for 24 hours before RNA was extracted for microarray gene expression analysis. A Causal Reasoning Engine, an internally developed platform that infers upstream molecular causes of experimental gene expression data in the context of prior biological knowledge, was deployed to analyze both the in vivo and in vitro datasets. Our results show that the mechanistic profiles in the two cell lines were similar for some compounds (Dobutamine and Ergocalciferol) but significantly different for others (Amiodarone and Amitriptylin). Interestingly, regardless of the similarities or differences in the cell lines we identified mechanisms that are translatable from in vivo to both cell lines (altered Rho/ROCK signaling) and mechanisms translatable to only one cell line (endoplasmic reticulum stress in H9C2 only). We also identified potentially measurable toxicity mechanisms for compounds with in vivo cardiotoxicity but failed to induce any obvious cellular injury in vitro that otherwise would have allowed for a prediction of toxicity (Dexamethasone). In conclusion, this study enhances our ability to develop truly predictive in vitro cardiac safety assessment assays by pursuing mechanisms that reflect in vivo biology in the proper candidate cell-based model.

PS 148 ASSOCIATION NETWORKS AND VISUALIZATION TOOLS TO CORRELATE PRECLINICAL SIGNALS TO DRUG-INDUCED NAUSEA IN MAN.

J. Glab[1], M. Clark[2], J. Valentin[1] and L. Ewart[1]. [1]*Safety Pharmacology, AstraZeneca, Alderley Park, United Kingdom and* [2]*Discovery Information, AstraZeneca, Wilmington, DE.*

The therapeutic value of many drugs can be limited by gastrointestinal (GI) adverse effects such as nausea and vomiting. These events can limit drug absorption, cause dose-limiting toxicity, affect patients' compliance and result in labeling restriction or ultimately drug discontinuation / withdrawal. Drug-induced nausea in Phase I studies has been quantified in 113 candidate drugs and was present in ~30% (Ewart et al, 2010). It is a multi-system reflex and a subjective human sensation and there may not be an analogous experience in animals. Hence little is known about preclinical biomarkers that may accurately and effectively predict drug-induced nausea in man. The aim of this analysis was to data mine published documents from MEDLINE and FDA Adverse Event Reporting System to identify preclinical GI effects that may be associated with nausea and that could be of potential use in its prediction. A total of 160 marketed drugs were found that caused nausea in man and were also reported to have plausible GI observations in animals. A visual representation was used to display the vast network of associations between the drugs and observations in the pre-clinical studies. Collectively, gastrointestinal ulcers were the most common observations in animals occurring in 40% of the drugs that caused nausea. Within this group, observations of stomach ulcers accounted for 59%. Other common GI effects observed in animals included: gastric lesions (17%), diarrhea (15%), changes in gastric acid secretion (14%) and emesis (12%). This investigation demonstrates the feasibility of data-mining approaches to facilitate discovery of novel, plausible associations that can be used to understand drug-induced adverse events. Utilizing existing public and/or proprietary data sets negates the need for additional animal usage. The next step would be to test this approach for its ability to predict drug-induced nausea.

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PS 149 DDT AND TCDD INTERACT WITH PROTEINS INVOLVED IN THE INSULIN RECEPTOR SIGNALING.

M. Cabarcas-Montalvo, D. Montes-Grajales and J. Olivero-Verbel. *Environmental and Computational Chemistry Group, University of Cartagena, Cartagena, Colombia.*

TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) and DDT (1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane) are toxic and persistent environmental pollutants that have been proposed as etiologic factors for diabetes. However, the existence of target proteins belonging to the insulin receptor signaling remains elusive. Accordingly, it has been hypothesized that some proteins involved in this pathway could be theoretical targets for DDT and its derivatives, as well as for TCDD. Reported proteins to be part of transduction mechanisms activated by insulin were obtained from Protein Data Bank (PDB), and prepared employing Sybyl 8.1. Autodock Vina was utilized for docking calculations between the energy-minimized geometries (ab initio) of organochlorine compounds and proteins, and LigandScout was used to identify ligand-residue interactions. The proteins with the highest binding affinities for both groups of compounds were different. Phosphodiesterase 10A (PDB 2wey) and the eukaryotic translation initiation factor 4E (eIF4E, PDB 1wkw) presented the greatest binding affinity scores for DDT derivatives (8.3-8.6 Kcal/mol), whereas in the case of TCDD, best values were computed for glycogen synthase kinase 3 beta (PDB 1uv5, -8.10 Kcal/mol) and glucocorticoid-regulated kinase 1 (PDB 3hdn; -8.10 Kcal/mol). These theoretical target proteins for DDT or TCDD are involved in different actions regarding insulin receptor activation, such lipolysis, protein synthesis, apoptosis, glycogen and fatty acids synthesis, and sodium transport, among others. Most protein-ligand complexes included hydrophobic and aromatic interactions, and in the case of eIF4E, the binding pocket is shared by several DDT-related compounds. In short, in silico studies have shown that DDT and TCDD have the theorethical ability to bind proteins involved in insulin signaling, and this could eventually influence the development of diabetes. Colciencias-Universidad de Cartagena (Grant 110745921616).

PS 150 COMPUTATIONAL BASED APPROACHES FOR IDENTIFYING AND UNDERSTANDING KINASES ASSOCIATED WITH CARDIOTOXICITY.

D. Puppala[1], V. Bonato[2], K. Leach[1], S. Louise-May[1], K. J. McConnell[3], M. Gosink[4] and M. T. Pletcher[1]. [1]*Compound Safety Prediction, Pfizer Inc., Groton, CT,* [2]*Biostatistics Group, Pfizer Inc., Groton, CT,* [3]*Computational Science CoE, Pfizer Inc., Groton, CT and* [4]*DSRD, Pfizer Inc., Groton, CT.*

Kinase biology is a complex network which offers promising drugs targets but also has been associated with significant adverse events like cardiotoxicity. The goal of this project was to identify kinases that have the greatest potential to induce cardiotoxicity if targeted and to understand the mechanisms by which they would induce that toxicity so that these adverse events could be predicted and avoided. Towards this end we first used a combination of different bioinformatics approaches; mining the mouse knockout, OMIM, and Toxreporter databases and employing an automated literature search tool. We then collated these results in order

to rank the kinases by an estimated relative risk. Based on this initial assessment, compounds targeting kinases like KDR, ABL1, MAPK8, PRKCA, p38 alpha, EGFR, ERBB2, MYLK, RAF1 and Tie2 would confer significant risk of inducing cardiotoxicity. Next to identify appropriate cell-based system would for confirming computational predictions of toxicity risk, we looked at basal expression levels of all the kinases across 253 human cancer lines. The entire normalized data (MAS5) was obtained from caArray database for further analysis. Of the 506 kinases, there are 70 kinases which are not expressed (average signal intensity<100) in any cell line and there are 29 kinases that are expressed (average signal intensity >100) across all cell lines. Of the ten kinases that have been associated with cardiotoxicity, KDR, TEK, MYLK and ERBB2 have been expressed in less than 75 % of cells lines suggesting that potential toxicity of kinase inhibitors that target these kinases would be missed in the majority of cell-based models. In conclusion, effective tools for predicting cardiotoxic risk of kinase inhibitors are desperately needed. This assemblage of knowledge and expression data provides the tools to estimate risk and then test that prediction in the most appropriate system.

PS 151 DISCOVERY OF NOVEL MICRO-RNAS FROM RAT LYMPHOCYTES DURING GLUCOCORTICOID INDUCED APOPTOSIS.

R. Shah[1], S. Vasa[1], L. Smith[2] and J. Cidlowski[2]. *[1]SRA International, Durham, NC and [2]National Institute of Environmental Health Science, Research Triangle Park, NC.*

miRNAs are post-transcriptional regulators and play a significant role in the normal functioning of eukaryotic cells. Aberrant expression of miRNA is known to be associated with many diseases including cancer, heart disease and psychological disorders. Recently, microRNAs have emerged as effective biomarkers of disease and toxic injury. This has generated a great deal of interest in identification and characterization of novel miRNAs in genomes of various species. The glucocorticoid receptor is a sensor of environmental stress and endogenous glucocorticoids influence the response of the immune system to environmental stress and toxic insult. Therefore, understanding the mechanisms governing glucocorticoid-induced apoptosis of lymphocytes is an important contribution to toxicological research. In this study, we use glucocorticoid-induced apoptosis of lymphocytes as a model system along with deep sequencing technology to investigate hormone dependent changes in miRNA expression. We use RNA preparation from untreated and glucocorticoid treated Rat primary thymocytes and employ small RNA sequencing protocol along with the Illumina-Solexa deep sequencing platform to obtain millions of short sequence reads. The sequencing data is analyzed using a comprehensive bioinformatics approach to calculate changes in expression of both previously annotated as well as potentially novel miRNAs. For novel miRNA discovery, we use a multi-step computational approach based on miRNA prediction algorithms using sequence features, secondary structure prediction and machine learning methods. Our results indicate that a large number of known miRNAs are down regulated up on hormone treatment. In addition, we find a set of potentially novel miRNAs whose expression also changes due to hormone treatment. Quantitative PCR validation agrees with our bioinformatics analyses for a set of putative novel miRNA loci, thus providing further evidence for the presence of novel miRNAs in the Rat lymphocyte transcriptome during glucocorticoid induced apoptosis.

PS 152 AUTOMATIC *IN SILICO* ASSESSMENT OF GENOTOXIC POTENTIAL IMPURITIES.

A. Brigo, G. Gerebtzoff, M. Kansy, T. Weiser and T. Singer. *Non-Clinical Safety, F. Hoffmann-La Roche Ltd., Basel, Switzerland.*

The synthesis of Active Pharmaceutical Ingredients (API) frequently involves the use of reactive reagents forming intermediates and by-products, which may still be present in the final drug substance as impurities. A subset of these impurities could present a potential for genotoxicity and carcinogenicity, posing an additional safety concern to patients and clinical subjects.
The EMEA Guideline and the FDA Draft Guidance Document prescribe that a safety expert shall evaluate the potential genotoxic activity of all solvents, intermediates, reactants and readily predicted side products within the synthetic route of each API, using in silico systems. Depending on the in silico classification and on the available experimental data, potential genotoxic impurities may require different follow-up actions: (i) performance of the Ames assay; (ii) chemical reasoning to exclude their presence (e.g. chemical instability); (iii) development of analytical methods to ensure that specific genotoxic impurities do not exceed a defined threshold of toxicological concern (TTC). Within the pharmaceutical industry, such activities require the coordination of many functions, including process chemistry, genetic toxicology and analytics. In order to further optimize the entire process and reduce any possible redundancies, an internal web-based system has been developed. On one hand, it allows chemists and toxicologists to interact more efficiently; on the other hand, it incorporates a centralized database - fully dedicated to genotoxic impurities - which describes: (i) the results of in silico assessments; (ii) Ames test results; (iii) whether or not a specific analytical method has been developed; (iv) project details; (v) if available, literature references. The introduction of this web-based tool halved the average time of in silico assessments of potential genotoxic impurities and completely eliminated the risk of any inefficient duplication of testing, method developments and assessments.

PS 153 AUTOMATED IDENTIFICATION OF STRUCTURE-TOXICITY RELATIONSHIPS USING ATOM SIGNATURES.

L. Carlsson, E. Ahlberg Helgee and S. Boyer. *Computational Toxicology, AstraZeneca, Mölndal, Sweden.*

Drug discovery is dependent on warning systems that use structural alerts to notify medicinal chemists of potential risks. One of the original uses of structural alerts was for mutagenicity. The derivation of these alerts have been traditionally manual and to some extent, subjective. We present a novel and fully objective method to efficiently mine chemical data for substructures. The method deterministically grows substructures from all atoms in the data using the signature descriptor. Each grown substructure is evaluated for significance using a p-value (distribution of the data containing the substructure with respect to the activity compared to the overall activity distribution), an accuracy and a minimum occurrence threshold. When a significant substructure is found the search for substructures is terminated in that direction. The results show that this method can retrieve significant substructures quickly and that they can be used to make accurate predictions, with substructures for both active and inactive fragments. The results are compared to existing methods on the following criteria: ability to capture information compared to manual curation, ability to predict new compounds and computational efficiency. The workflow is demonstrated using public AMES mutagenicity data and has been compared to the hand-curated toxicophores reported in the literature. The results show that the accuracy of the proposed method is on par with hand curation of toxicophores. The proposed method signals both for positive and negative compounds, and the significant substructure extraction from the data takes roughly 20 minutes. The proposed method has also been compared to clique based methods, which are by far more computationally intensive. In cases where the clique based methods generates a million substructures the proposed method generated roughly one thousand but with retained predictive accuracy.

PS 154 COMPUTATIONAL ASSESSMENT OF REACTIVE METABOLITE RISK IN DRUG DISCOVERY USING MECHANISTIC ENSEMBLE MODELLING.

S. Boyer[1], C. Hasselgren[1], L. Peterlin-Masic[2], O. Spjuth[3] and L. Carlsson[1]. *[1]Computational Toxicology, AstraZeneca, Mölndal, Sweden, [2]Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia and [3]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden.*

The formation of reactive metabolites is considered an unnecessary safety risk in drug discovery. To minimize this risk early drug design methods are required that both alert for potential reactive metabolite hazard, but also of the probability that it will actually be formed. This output would be generated from the chemical structure without the necessity of synthesis and experimental testing. The formation of reactive intermediates depends on a number of factors: 1) the presence of a chemical structure that will yield a chemically reactive species upon biotransformation 2) the probability that a metabolic event will actually occur in that particular structure 3) the metabolic rate of the molecule will be such that enough of the reactive species will be formed to pose a risk. This combination of factors makes the use of a simple QSAR model impractical. Instead we have employed an ensemble approach to the problem by using computational tools to address the above factors individually and then combined them to assess, using actual reactive metabolite screening data, the conditions dictated by the individual tools that will indicate high and low risk for reactive metabolite formation. Assessment of hazardous substructures is by SMARTS matching in a database of examples from literature and in-house data. The probability of biotransformation within that substructure is preformed using MetaPrint2D. Finally, the rate of metabolism is assessed using physico-chemical properties, QSARs or actual biotransformation rate data. The results of the validation against suggests a set of conditions that if met, predict positive and negative trapping results with greater than 80% accuracy. These results

suggest that the combination of tools to address reactive metabolite risk can provide design teams with an assessment of real risk rather than the simple hazard alert of a structural alert.

PS 155 DESIGN AND DEVELOPMENT OF AN INSTITUTIONAL KNOWLEDGE-BASE AT FDA'S CENTER FOR FOOD SAFETY AND APPLIED NUTRITION.

K. Arvidson, A. McCarthy, C. Yang and D. Hristozov. *CFSAN, U.S. FDA, College Park, MD.*

The Chemical Evaluation and Risk Estimation System (CERES) project under development in FDA's Center for Food Safety and Applied Nutrition aims at establishing a sustainable data management and storage system that will provide decision support tools for both pre-market and post-market safety assessments of food additives and food contact substances as well as for potential contamination issues. The development of CERES will provide a single unified data repository that compiles available information on a substance, including: chemical structures and properties, regulation records, toxicity studies, and other biological screening assays. In cases where no information is available for a particular substance, CERES provides tools to identify potential safety concerns by applying mode of action-driven QSAR prediction models as well as to identify and analyze data on structural and biological analogs (read-across). To achieve this goal, CERES requires a solid foundation for handling chemical and toxicity data as well as knowledge (e.g., rules or computational toxicology models) derived from these data. Thus the CERES project not only needs to address a wide variety of issues in database and knowledgebase construction, but also in the development of computational methods. Strategies are presented for tasks of data harvesting, data modeling, development of rule-base and computational models. This poster will focus on the overall strategy for the CERES knowledgebase; each sub-area will also be presented in other posters.

PS 156 PHARMACOLOGICAL PROFILE SIMILARITY – A RELEVANT METHOD FOR ANTICIPATION OF *IN VIVO* SAFETY?

D. Muthas[1], M. Rolf[2], S. Matis-Mitchell[3] and S. Boyer[1]. [1]*Global Safety Assessment, AstraZeneca R&D, Mölndal, Sweden,* [2]*Global Safety Assessment, AstraZeneca R&D, Alderly Park, United Kingdom and* [3]*Discovery Information, AstraZeneca R&D, Wilmington, DE.*

We have analyzed a pharmacological profile of historical AstraZeneca development compounds in order to investigate their potential to predict in vivo findings. A panel of 100 targets, most of which have been chosen for their association with adverse effects, was used to derive a pharmacological 'biospectrum' of each compound. The similarities of these biospectra were then calculated using several different similarity metrics (e.g. Tanimoto, Dice, Yule, Euclidian distance and Mahalanobis distance) and activity binning to identify compounds sharing similar biological fingerprints. To evaluate their relevance, these similarities were then compared to the preclinical and/or clinical findings of the compounds found by mining preclinical study reports and clinical safety databases. The observations were later analyzed using all found data as well as with special focus on certain types of studies (eg 1 month dog and 2 week rat). The compounds were then clustered based in the in vivo effects and compared to the clusters based on biospectrum. The results of these studies indicate that several pairs of structurally dissimilar compounds with similar biospectra can be identified and an overlap of their preclinical findings was frequently found. The frequency of association of the biospectrum and outcome was assessed for statistical significance and found to be significant in several cases. This suggests that pharmacological profiling and establishing a biospectrum could help identify the potential for safety issues from more than the chemical structure or the activity on a single target.

PS 157 DEVELOPMENT OF QSAR MODEL FOR HIGH-SPEED *IN SILICO* IDENTIFICATION OF POTENTIALLY PHOTOTOXIC ORGANIC COMPOUNDS.

W. Plonka[1] and J. M. Ciloy[2]. [1]*Life Science, FQS Poland, Cracow, Poland and* [2]*Life Science, Fujitsu Kyushu Systems Ltd., Fukuoka, Japan.* Sponsor: K. Hayamizu.

Light-exposure induced toxicity to humans is one of factors limiting the use of chemical compounds in wide range of applications, starting with cosmetics, household chemistry products, as well as pharmaceutical industry. The possibility of reliable in-silico identification of potentially phototoxic compounds would contribute to product safety and reduce development costs. A Qualitative Structure-Activity Relationship study had been done on a set of 229 structures, of which 114 were known from public literature to cause phototoxic effects, and the rest were neutral compounds, including compounds routinely used in commercial cosmetics. The objective was to create a model that would have as low as possible false-negative classification rate to ensure high safety margin of the predictions. It was assumed, that a molecule has to satisfy a set of quantum, as well as structural properties – to which both light absorption and energy transfer in biological systems relate in order to be potentially phototoxic. A stochastic gradient perceptron model was created employing quantum descriptors calculated by fast AM1 semiempirical method, fragment count and topological descriptors. The model has a 0% false-negative classification rate on the training set. The overall internal leave-1-out cross validation rate of the model is over 90%. The details of model building technique and compounds used in research are presented in the poster.

PS 158 THE POWER OF AN ONTOLOGY-DRIVEN DEVELOPMENTAL TOXICITY DATABASE FOR DATA MINING AND COMPUTATIONAL MODELING.

A. Richard[1], E. Julien[2], E. Busta[3], M. Wolf[4] and C. Yang[3]. [1]*NCCT (D343-03), U.S. EPA, Research Triangle Park, NC,* [2]*LifeSci Research Service, Rockville, MD,* [3]*HFS-2775, U.S. FDA CFSA, College Park, Maryland, MD and* [4]*Lockheed-Martin, U.S. EPA, Research Triangle Park, NC.*

Modeling of developmental toxicology presents a significant challenge to computational toxicology due to endpoint complexity and lack of data coverage. These challenges largely account for the relatively few modeling successes using the structure–activity relationship (SAR) paradigm. The development of new in vitro profiling approaches, employing screening assays or lower organisms to evaluate developmental toxicity requires anchoring to the results of in vivo studies. The International Life Science Institute (ILSI) recently released a public ontology-driven DevToxDB with data extracted from published studies. This project heavily influenced other efforts, and the data content in the ILSI DevToxDB was combined into an ontology-based database along with other public data from EPA ToxRefDB, FDA Center for Food Safety and Applied Nutrition and Center for Drug Evaluation Research and the National Toxicology Program. All chemical structures are indexed in DSSTox, providing the ability to assess chemical coverage and diversity of these largely non-overlapping data inventories, which include drugs, pesticides, industrial chemicals, food ingredients and contact substances. The use of a common toxicological ontology provides a logical means to group and aggregate biological effects in subsequent computational analyses. Chemoinformatics methods are used to compare the chemical space of each data source, and to explore associations with biological endpoints through the toxicology ontology perspective. Chemicals in these distinct datasets cause common phenotypes as well as distinct, non-overlapping phenotypes in the same target organs. Incorporation of the ILSI DevTox database into other public database efforts should provide a rich foundation for spurring innovation in SAR modeling of developmental endpoints. This abstract does not necessarily represent policies of FDA or EPA.

PS 159 USE OF *C. ELEGANS* GROWTH ASSAY DATA IN NOVEL TWO-STEP HIERARCHICAL QSAR MODELING WORKFLOW ENHANCES *IN VIVO* TOXICITY PREDICTION FOR TOXCAST PHASE I CHEMICALS.

A. Golbraikh[1, 2], R. Shah[1, 4], W. Boyd[3], M. Smith[4], A. Tropsha[1, 2] and J. Freedman[3]. [1]*SciOme LLC, Research Triangle Park, NC,* [2]*University of North Carolina at Chapel Hill, Chapel Hill, NC,* [3]*National Institute of Environmental Health Science, Research Triangle Park, NC and* [4]*SRA International, Durham, NC.*

The US EPA ToxCast Phase I program provides data for 320 substances with known in vivo toxicity measured in 76 assays; the results of ca. 600 in vitro assays for the same substances are available as well. The latter data have been used previously with varying degree of success to improve the predictive power of in silico models of chemical toxicity. Herein, we have explored, in the same context, new whole organism toxicity data generated for ToxCast chemicals in the C. elegans growth assay. The goal of this study was to establish both the absolute as well as relative (i.e., with respect to other short term assays included in the ToxCast Phase I database) value of C. elegans growth assay for predicting chemical in vivo toxicity and prioritizing new chemicals for in vivo studies. Unlike most in vitro assays where the number of active compounds was typically lower than that of inactive ones, the C. elegans assay resulted in nearly balanced dataset. kNN QSAR modeling of C. elegans data using our standard computational workflow with the emphasis on external validation resulted in models with the total accuracy of 66%. The C. elegans

data were further explored in the following novel hierarchical QSAR modeling workflow. First, all chemicals were partitioned into two classes based on whether a compound tested similarly (Class I) or dissimilarly (Class 2) in both C. elegans and three mouse in vivo assays resulting in 96, 109, and 117 of Class I and 117, 112, and 100 Class II compounds. Second, each in vivo end point classification models to distinguish toxic vs. non-toxic compounds within each Class were developed using kNN binary QSAR approaches and the total external accuracy was in the range of 68-71% exceeding that of the same models generated with chemical descriptors only or using other ToxCast in vitro data.

PS 160 EVALUATION OF JP-8 JET FUEL INDUCED HEARING LOSS IN RATS.

L. D. Fechter[1], J. W. Fisher[2], G. D. Chapman[3], V. P. Mokashi[3], J. E. Reboulet[3], J. E. Stubbs[4], A. M. Lear[3], S. L. Prues[3], C. A. Gearhart[1], S. Fulton[1] and D. R. Mattie[5]. *[1]Jerry Pettis Memorial VA Medical Center, Loma Linda, CA, [2]U.S. FDA/NCTR, Jefferson, AR, [3]NHRC/EHEL, Wright-Patterson Air Force Base, OH, [4]AFIT/ENV, Wright-Patterson Air Force Base, OH and [5]711 HPW/RHPBA, Wright-Patterson Air Force Base, OH.*

Noise-induced hearing loss (NIHL) continues to be a major military operational problem and general occupational health hazard. Simultaneous or successive exposure to noise and specific chemical agents can potentiate NIHL or produce additive effects. Studies (28-day) with male and female rats were designed to study combined JP-8 jet fuel and noise effects on auditory function. An initial study identified subchronic noise exposure that yielded a NOAEL, LOAEL, and significant NIHL. Rats were exposed to 0, 75, 85 or 95 dB for 6 h per day, 5 days per week over 4 wk. For noise exposure, audio editing software was used to filter and equalize a white noise file to one octave-band wide, centered at 8 KHz. Signal was split into three equalizers and amplifiers for producing the three noise levels generated using electrodynamic shakers mounted to exposure chambers. Hearing loss was assessed using the distortion product otoacoustic emission (DPOAE) test to evaluate outer hair cell function and the compound action potential (CAP) test to determine hearing threshold. Histological examination of cochlea was conducted to determine the percentage of hair cell loss. All data from the first study showed significant permanent impairment of hearing at 95 dB and a NOAEL at 75 dB. Noise exposure of 85 dB was identified as LOAEL and was used in the second study to investigate combined effects of jet fuel and noise on hearing. Rats were exposed to 85 dB and either 200, 750 or 1500 mg/m^3 JP-8 for 6 h per day, 5 days per wk over 4 wk. DPOAE, CAP and histology of cochlea for rats exposed to noise and JP-8 showed a dose response increase in hearing loss greater than seen with just 85 dB alone in the first study. Next we will examine effects of JP-8 alone on NIHL to confirm potentiating effect of jet fuel. Supported by AF/SGRS and LLVAMC.

PS 161 1, 3-DICARBONYL ENOLATES: A NEW CLASS OF NEUROPROTECTANTS.

R. M. LoPachin[1], T. Gavin[2], D. Casper[3] and D. S. Barber[4]. *[1]Department of Anesthesia Research, Montefiore Medical Center, Bronx, NY, [2]Chemistry, Iona College, New Rochelle, NY, [3]Neurosurgery, Montefiore Medical Center, Bronx, NY and [4]CEHT, University of Florida, Gainesville, FL.*

Curcumin, phloretin and structurally related phytopolyphenols have well-described neuroprotective properties that appear to be at least partially mediated by 1,3-dicarbonyl enol substructures that form nucleophilic enolates. Based on their structural similarities, we tested the hypothesis that enolates of simple 1,3-dicarbonyl compounds such as acetylacetone might also possess neuroprotective actions. Our results show that the 1,3-dicarbonyls, particularly 2-acetylcyclopentanone (2-ACP), protected rat striatal synaptosomes and a neuronal cell line (MN9D) from thiol loss and toxicity induced by acrolein, an electrophilic α,β-unsaturated aldehyde. The 1,3-dicarbonyl compounds also provided substantial cytoprotection against toxicity induced by hydrogen peroxide in a cellular model of oxidative stress. Initial chemical characterization in cell-free systems indicated that the 1,3-dicarbonyl compounds acted as surrogate nucleophilic targets that slowed the rate of sulfhydryl loss caused by acrolein. Although the selected 1,3-dicarbonyl congeners did not scavenge free radicals, metal ion chelation was a significant property of both 2-ACP and acetylacetone. Oral 2-ACP (100-300 mg/kg/d x 10d) did not cause weight loss or overt toxicity in treated Sprague-Dawley rats (300-325g). In an animal model of oxidative stress (carbon tetrachloride; 50 mg/kg, i.p.), pretreatment (6 days) with oral 2-ACP (200 mg/kg) significantly reduced markers (plasma ALT activity) of liver injury by 36%. Our data suggest that the 1,3-dicarbonyl enols represent a new class of neuroprotectants that scavenge electrophilic metal ions and unsaturated aldehydes through their nucleophilic enolate forms. As such, these enols might be rational candidates for treatment of acute or chronic neurodegenerative conditions that have oxidative stress as a common molecular etiology. Sponsored by NIEHS grant ES03830-24.

PS 162 MOLECULAR MODELING OF 1, 3-DINITROBENZENE (1, 3-DNB) INTERACTIONS WITH ADENOSINE DEAMINASE (ADA).

R. J. Richardson[1], S. J. Wijeyesakere[2], Y. Wang[1] and M. A. Philbert[1]. *[1]Department of Environmental Health Sciences, Toxicology Program, University of Michigan, Ann Arbor, MI and [2]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI.*

1,3-DNB produces a selective, focal edematous lesion in brainstem astrocytes 24 h after oral administration to adult male rats. The mechanism of this toxicity is unknown, but recent work in our laboratory has shown that the zinc-containing enzyme ADA is inhibited by 1,3-DNB with an IC50 of 287 μM. Inhibition curves exhibited a steep Hill slope of 5.6, suggesting the possibility that 1,3-DNB could be an aggregating inhibitor. Standard tests for aggregation were negative, but dynamic light scattering studies showed that 1,3-DNB in a concentration range of 137-805 μM formed particles of 41-190 nm mean diameter. Kinetics results were consistent with uncompetitive inhibition, but other models are possible. In order to provide additional insights into possible self-assembly of 1,3-DNB molecules into aggregates and the inhibition of ADA by 1,3-DNB, molecular dynamics (MD) simulations were carried out by YASARA-Structure 10.9.17 and docking studies were carried out with AutoDock 4.2.3. MD studies undertaken with 0.60 M DNB in a (90 Å)3 box of water showed that it forms coplanar stacks consisting of 2 to 6 molecules per stack. Docking studies found that the most energetically favorable conformation (-7.2 kcal/mole; predicted Ki = 5.05 μM) involved 1,3-DNB occupying the active site of ADA with each oxygen of one nitro group in close proximity (1.80 Å) to the zinc ion. However, 4 other clusters occupying peripheral sites were found with lowest energy conformations ranging from 5.1 to -4.9 kcal/mole; predicted Ki = 195 to 242 μM. The results suggest that 1,3-DNB forms molecular aggregates and that the monomer binds to the active site as well as peripheral sites on ADA. Further work will be required to determine if aggregates of 1,3-DNB can function as inhibitors and if peripheral binding sites are involved in allosteric inhibition. Supported by NIH RO1 ES008846.

PS 163 1, 3-DINITROBENZENE (1, 3-DNB) INHIBITS ADENOSINE DEAMINASE (ADA) LEADING TO INCREASED ADENOSINE LEVELS IN IMMORTALIZED DI TNC-1 ASTROCYTES.

Y. Wang[1], X. Liu[2], B. Schneider[1], E. A. Zverina[4], K. A. Russ[1], C. A. Fierke[3], R. J. Richardson[1] and M. A. Philbert[1]. *[1]Department of EHS, Toxicology, University of Michigan, Ann Arbor, MI, [2]Department of Chemistry, University of Michigan, Ann Arbor, MI, [3]Department of Biological Chemistry, University of Michigan, Ann Arbor, MI and [4]Chemical Biology Program, University of Michigan, Ann Arbor, MI.*

In the manufacture of dyes, plastics, and explosives, 1,3-DNB is used as an intermediate. Pathologic analysis reveals that 25 mg/kg 1,3-DNB p.o. in male rats for 24 h induces a selective, focal edematous lesion in brainstem astrocytes. The mechanism of 1,3-DNB toxicity in astrocytes is still elusive. We propose that 1,3-DNB inhibits ADA, leading to increased adenosine concentrations [Ado]. Eventually, increased [Ado] induce astrocyte apoptosis. To test this hypothesis, ADA activity and [Ado] were characterized before and after 1,3-DNB treatment of DI TNC-1 astrocytes in vitro. ADA activity in the presence of [1,3-DNB] ranging from 0 μM to 1000 μM was measured spectrophotometrically. [Ado] and inosine [Ino] were detected in conditioned media using Ado and Ino sensors. 1,3-DNB inhibited ADA activity in a concentration-dependent manner with an IC50 of 287 μM. Consistent with decreased ADA activity, [Ado] increased to 120% and 164% of control at 2 and 4 h after exposure to 1 μM 1,3-DNB, whereas [Ino] decreased to 69% and 58% of control. Native gel electrophoresis showed that 1,3-DNB was not an irreversible denaturant of ADA. In further tests for aggregates, adding 3 different concentrations (w/v) (0.01%, 0.02%, 0.05%) of Triton X-100, or BSA 0.1 mg/ml into the reaction system, or changing [ADA] (0.5 nM and 1.5 nM) did not alter the IC50 of 1,3-DNB. However, dynamic light scattering showed particles 41-190 nm in diameter with 137-805 μM 1,3-DNB. Although kinetics studies were consistent with uncompetitive inhibition of ADA by 1,3-DNB, with Km = 55 μM, Ki = 203 μM, and n = 2.9, other models are possible. Further work involving molecular modeling will be carried out to provide added insight into the mechanism of inhibition of ADA by 1,3-DNB. Supported by NIH RO1 ES008846.

PS 164 TRANSLOCATOR PROTEIN (18 KDA) (TSPO): A PRE-CLINICAL BIOMARKER OF NEURODEGENERATION IN SANDHOFF DISEASE MICE.

J. Choi[1], H. Wang[2], C. J. Endres[2], J. J. Fox[2], G. Green[2], M. G. Pomper[2] and T. R. Guilarte[1]. [1]*Environmental Health Sciences, Columbia University Mailman School of Public Health, New York, NY and* [2]*Radiology, Johns Hopkins Hospital, Baltimore, MD.*

Translocator protein (18 kDa) (TSPO), formerly known as the peripheral benzodiazepine receptor (PBR), has been widely used as a sensitive biomarker of brain injury and inflammation. Sandhoff disease is an autosomal recessive neurodegenerative disease characterized by excess glycolipid storage due to the lack of lysosomal β-hexosaminidase. A deficiency of this enzyme results in impaired degradation of G_{M2} and G_{A2} gangliosides and other glycolipids, leading to severe neurodegenerative changes in the brain. In the present study, we used a mouse model of Sandhoff disease to assess the longitudinal expression of TSPO as a function of disease progression. Using a novel TSPO-specific ligand [^{125}I]Iodo-DPA-713, we found that TSPO levels were significantly increased in relevant brain regions prior to the behavioral expression of disease using *ex vivo* quantitative autoradiography. These findings were confirmed using *in vivo* [^{125}I]Iodo-DPA-713 microSPECT imaging. We also show that the increase in TSPO levels was associated with neurodegenerative changes using silver staining and activation of microglia and astrocytes visualized by immunohistochemistry. In brain regions known to be undergoing neurodegenerative changes, there was differential expression of microglia and astrocytes with microglia being activated early in the neurodegenerative process and astrocytes activated at a later time point. These findings provide strong evidence that TSPO can be used as an early pre-clinical biomarker of neurodegenerative changes prior to the behavioral manifestation of disease. Further, the novel TSPO radioligand [^{125}I]Iodo-DPA-713 appears to be a useful SPECT ligand for the quantitation and visualization of TSPO levels in the rodent brain.

PS 165 THE CYANOBACTERIAL NEUROTOXIN β-N-METHYLAMINO-L-ALANINE (BMAA) INDUCES UNFOLDED PROTEIN RESPONSE (UPR) AND CHANGES OF SOD ACTIVITY LEVELS IN HUMAN NEURONAL CELLS.

O. Okle[1], M. Helmer[1], K. Stemmer[1, 2] and D. R. Dietrich[1]. [1]*Human & Environmental Toxicology, University of Konstanz, Konstanz, Germany and* [2]*Obesity Research Centre at the Metabolic Disease Institute, University of Cincinnati, Cincinnati, OH.*

The cyanobacterial β-N-methylamino-L-alanine (BMAA) is described as an excitotoxin and assumed to be involved in the development of amyotrophic lateral sclerosis/ Parkinsonism-dementia complex (ALS/ PDC) on Guam. Although potential biomagnification of BMAA in the food chain of the indigenous Chamorro of Guam may lead to the observed ALS/ PDC, BMAA was also reported outside of the Pacific area, i.e. in brain tissue of Canadian patients with Alzheimer's disease.
The toxic effect of BMAA in in vivo and in vitro studies, mouse and rat models, is primarily characterized by its acute high dose excitotoxicity which, however, does not explain the chronic exposure associated effects e.g. ALS/ PDS. It is therefore plausible that in the observed human neuropathy intracellular mechanisms, other than excitotoxicity, are involved in the progressive degradation of motor neurons.
Within 3 hours of exposure of the human neuroblastoma cell line SH-SY5Y, BMAA induced an unfolded protein response (UPR) and an alteration of additional stress response proteins. A dose and time dependent increase of ROS, formerly detected in neuronal rodent models, was also observed in this human neuronal model. Increased ROS was also linked to a modified expression and activity of SOD1. As changes in SOD activity are closely related to the pathogenesis of familial ALS patients, both, changed SOD activity and UPR, as observed in the human neuronal cell model, could provide for a slow demise of motor neurons and thus contribute to the onset or progression of ALS/PDC in humans.

PS 166 CYTOTOXICITY OF β-N-METHYLAMINO-L-ALANINE (L-BMAA) AND METHYLAZOXYMETHANOLACETATE (MAMAC) AND ASSOCIATED DIFFERENTIAL MRNA EXPRESSION IN HUMAN NEURONAL CELLS.

M. Helmer[1], O. Okle[1], K. Stemmer[1, 2] and D. R. Dietrich[1]. [1]*Human & Environmental Toxicology, University of Konstanz, Konstanz, Germany and* [2]*Obesity Research Centre at the Metabolic Disease Institute, University of Cincinnati, Cincinnati, OH.*

The cyanobacterial toxin β-N-methylamino-l-alanine (L-BMAA) is neurotoxic amino acid acting via excitotoxic pathways. The cycad plant toxin cycasin and its aglykon methylazoxymethanol (MAM) and acetate methylazoxymethanolacetate (MAMAc) have been described to be genotoxic via formation of DNA adducts. There is a possible link between the consumption of cycas plants containing both toxins and the high incidence of the neurodegenerative disease amyotrophic lateral sclerosis/ Parkinsonism dementia complex (ALS/PDC) among the indigenous Chamorro people on the Mariana Islands. The aim of the current in vitro study was to characterize the effects of L-BMAA and MAMAc on the growth, cell viability and mRNA expression in the human neuroblastoma cell line SH SY5Y.
We observed a time and dose dependent cytotoxicity of MAMAc, determined via the MTT reduction assay, neutral red uptake test and non-differential cell counts. The susceptibility of cells to L-BMAA cytotoxicity appeared to be increased by pretreatment of the cells with MAMAc. Exposure to non-cytotoxic concentrations of MAMAc and L-BMAA (increased/decreased) mRNA expression, examined by cDNA synthesis and qPCR, of the cellular stress markers O6-methylguanine-DNA methyltransferase (MGMT), 70 kd Heat shock Protein (Hsp70) and BCL2-associated athanogene 1 (BAG1). Thus cycasin and L-BMAA exposure could induce an increased dysfunctionality of cellular homeostasis in human neuronal cells and thus contribute to the onset or progression of ALS/PDC.

PS 167 PERTURBATION OF REGIONAL BRAIN METABOLISM IN F344 RATS AFTER LOW LEVEL EXPOSURE TO DIISOPROPYLFLUOROPHOSPHATE (DFP).

D. Mahle[1] and N. V. Reo[2]. [1]*AFRL/RHPB, Wright-Patterson Air Force Base, OH and* [2]*Wright State University, Fairborn, OH.*

The mechanism of organophosphate (OP) induced inhibition of acetylcholinesterase and subsequent excitotoxicity is well described. However, exposure to OPs at non-cholinergic doses has been reported to cause aberrations in neuronal development, cellular signaling and deficits in cognitive behavior and spatial memory. Little is known about the mechanisms of action for this noncholinergic toxicity of OPs. Using multinuclear NMR techniques the metabolic effects of DFP in rat brain can be investigated and possible correlations with acetylcholinesterase (AChE) inhibition can be explored. Adult male F344 rats were administered 1 mg/kg DFP or saline via subcutaneous injection at 10 mL/kg and were euthanized at 0.5, 1, 2, 12, 24 and 48 hr post dose. Brains were removed and cortex, cerebellum, hippocampus and brainstem were collected. Lipid and aqueous extracts were prepared from each brain region, and profiles of small molecule metabolites, lipids and phospholipids were measured using 1H, 13C and 31P NMR spectroscopy. Whole blood AChE activity in DFP-treated rats was 77% of control values at 2 hr post dose and continued to decline to 65% at 48 hr. All brain regions reached a minimum of AChE activity (40-55%) at 1-2 hr post dose with the exception of cortex which had a minimum of activity at 12 hr post dose. AChE activity returned to 60-80% of control by 48 hr. At 2 hr post dose, cortex showed the most changes in the lipid profile with significant decreases in cholesterol, ω-3 and ω-6 fatty acids, as well as phosphatidylcholine, phosphatidylethanolamine and ethanolamine plasmalogen. The mitochondrial phospholipid cardiolipin was significantly decreased after 2 hr in brainstem. Niacinamide was increased in brainstem and hippocampus 2 hr post DFP, while succinate was increased in cerebellum 2 hr post DFP. By evaluating the impact of low level OP exposure on the metabolic profile of specific brain regions, we hope to gain a greater understanding of the noncholinergic mechanisms of action and sensitive target areas of OPs.

PS 168 INVESTIGATING GENE-ENVIRONMENT INTERACTIONS RELEVANT TO PARKINSON'S DISEASE USING DROSOPHILA.

C. A. Remillard, H. O. Lawal and D. E. Krantz. *University of California, Los Angeles, Los Angeles, CA.*

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the selective loss of dopaminergic (DA) neurons. The vast majority of PD cases are sporadic and their etiology is poorly understood. Exposure to environmental toxins increases the risk of PD but individual susceptibility varies suggesting additional genetic effects. We are using Drosophila to investigate these potential gene-environment interactions. Since DA itself may be neurotoxic, genes that regulate cellular DA levels may be particularly important. We tested the effects of two genes that regulate levels of cellular dopamine and its metabolites: the Drosophila vesicular monoamine transporter (dVMAT), which packages and transports dopamine into synaptic vesicles, and Drosophila aldehyde dehydrogenase (dALDH), which converts the highly toxic dopamine metabolite 3,4dihydroxyphenylacetaldehyde (DOPAL) into the less toxic species 3,4dihydroxyphenyl acetic acid (DOPAC). We exposed dVMAT and dALDH mutant flies to pesticides, including paraquat and rotenone, both of which are established models of PD. We then assayed organismal survival, locomotor behaviors related to DA signaling and the number of DA neurons that remained

after pesticide exposure. We show that over-expression of dVMAT confers neuroprotection of DA cells against chronic exposure to rotenone but not paraquat and that dALDH mutant flies display a drastic reduction in lifespan when exposed to paraquat. Our findings indicate that select DA pathway genes may play a key role in determining susceptibility to environmental risk factors for PD and provide a useful model to investigate the mechanism by which environmental toxins and genes interact.

PS 169 IDENTIFYING LESS TOXIC TRIARYL PHOSPHATES FOR JET ENGINE LUBRICANTS.

P. E. Baker[1], T. B. Cole[1, 2], K. E. Thummel[3], Y. S. Lin[3], A. L. Co[1], A. E. Rettie[3], J. H. Kim[4] and C. E. Furlong[1]. *[1]Medicine, Medical Genetics, & Genome Sciences, University of Washington, Seattle, WA, [2]Environmental & Occupational Health Sciences, University of Washington, Seattle, WA, [3]Pharmaceutics and Medicinal Chemistry, University of Washington, Seattle, WA and [4]Anesthesiology & Pain Medicine, University of Washington, Seattle, WA.*

Due to their performance characteristics, triaryl phosphates (TAPs) continue to be used as jet lubricant additives, even though their neurotoxic nature has been known for at least 75 years. When engine seals fail, TAPs can enter aircraft via bleed air that provides air to the cabin, exposing passengers and aircrew. Some next-generation aircraft will bypass this issue by directly venting fresh air without passing through engines; but it will be decades until present-day planes are decommissioned, so aircraft exposures will remain a problem. One way to reduce consequences of exposures is to use less toxic TAPs. Our goal was to develop a means to rapidly detect potentially less toxic TAPs, using a biomarker of organophosphate exposure, butyrylcholinesterase (BChE). TAP screening involved 1) incubating candidate TAPs with human or rat liver microsomes and NADPH, and 2) quantifying resultant metabolites for BChE inhibition in a microassay. Initial screening of 20-plus TAP samples uncovered eight compounds that were non-inhibitory; two [tri-(p-tert-butylphenyl) phosphate, TpBP and tri-(p-cresyl) phosphate, TpCP] plus one proven-inhibitory TAP [Durad 125 (D125)] were used to expose mice by gavage. Acyl peptide hydrolase (APH), carboxylesterase (CES) and BChE levels were assessed from liver, plasma and/or RBCs 24 hrs later. Bioactivated-D125 inhibited all enzymes in every tissue assessed. TpCP was toxic to liver APH and CES, and plasma CES. While TpBP was somewhat toxic to plasma CES, it was significantly less than TpCP or D125; TpBP was not toxic to liver enzymes. Lack of inhibition of BChE by bioactivated TpBP suggests that the bioactivated metabolite did not reach the active site serine of BChE but did reach and inhibit the active site serine of CES, producing much less inhibition, however, than the other tested TAPs.

PS 170 LIPOPOLYSACCHARIDE INCREASES SODIUM INFLUX AND DOWN-REGULATES VOLTAGE GATED SODIUM CHANNEL MRNA EXPRESSION IN ACTIVATED MICROGLIA.

M. M. Hossain and J. R. Richardson. *Environmental and Occupational Medicine, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ.*

Although the specific causes of Parkinson's disease remains unknown, an abundance of evidence indicates that neuroinflammation and microglial activation leads to damage of striatal dopaminergic (DA) neurons. Microglia have been found to express a number of ion channels, including sodium channels (Na(v)), which appear to regulate multiple functions of these cells (Black et al., 2009). To determine the role of sodium and Na(v) in microglial activation, we examined sodium influx and mRNA expression of Na(v) isoforms in an immortalized mouse microglial cell line (BV-2) following lipopolysaccharide (LPS) exposure. BV-2 cells were activated by LPS (0-100 ng/ml) acutely or for 6 hr and sodium influx and mRNA expression of TNF-α and several Na(v) isoforms were measured. Acute administration of LPS (10 or 100 ng/ml) caused an immediate and prolonged 48%-60% increase in Na+ influx into BV-2 cells, which was abolished by Na(v) antagonist tetrodotoxin (TTX, 1μM). LPS increased TNF-α mRNA in a dose-dependent manner. However, TTX did not prevent the increase in TNF-α mRNA. Following 6 hr of exposure, LPS significantly decreased Na(v)1.2, Na(v) 1.3, Na(v) 1.4, Na(v) 1.6 and Na(v) 1.8 mRNA expression by 30-60% in a dose-dependent manner. Co-treatment of cells with TTX prevented effects of LPS at 10 ng/ml, but not at 100 ng/ml. Taken in concert, these data suggest that influx of sodium is an early event in microglial activation by LPS that leads to down-regulation of Na(v) isoforms, but does not appear to alter the induction of TNF-α mRNA. Supported by NIH ES015991 and ES005022.

PS 171 LIPOPOLYSACCHARIDE ACTIVATION OF MICROGLIA DIFFERENTIALLY REGULATES mRNA EXPRESSION OF ABC EFFLUX TRANSPORTERS.

C. J. Gibson[1], M. M. Hossain[2], J. R. Richardson[2] and L. Aleksunes[1]. *[1]Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and [2]Environmental and Occupational Medicine, University of Medicine and Dentistry of New Jersey, Piscataway, NJ.*

Efflux transporters are an important component of a cell's ability to export endogenous compounds as well as toxicants. Several efflux transporters of the ATP binding cassette (ABC) family located in neurons, astrocytes, microglia and within the blood-brain barrier function to limit local exposure to many chemicals. Alterations in the expression and activity of these transporters could potentially lead to pharmacokinetic changes for their substrates. This study quantified changes in mRNA expression of various efflux transporters in activated microglia. Immortalized mouse BV2 microglia cells were activated by exposure to lipopolysaccharide (LPS) at various concentrations (0, 1, 10, 100, 500 ng/ml) for 6 and 24 h. mRNA expression of inflammatory cytokines (TNFα, IL-1β, Cxcl-1, Cxcl-10) and transporters (Mdr1a, Mdr1b, Mrp1-5, Bcrp) was determined using quantitative PCR. Consistent with microglial activation, cytokine mRNA expression was up-regulated at 6 h after LPS and declined by 24 h. LPS treatment decreased Mrp2, Mrp3, and Bcrp mRNA expression by ~50% at 6 h in a dose-dependent manner. Conversely, Mrp1 and Mrp5 mRNA exhibited an 80% increase in expression. By 24 h, transporter mRNA expression (Mdr1a/1b, Mrp3, 4, and Bcrp) was reduced more than 50% with the exception of Mrp2 and 5 which were up-regulated by 100%. Determining how efflux transporters are regulated in microglia during inflammation may aid in understanding cell communication and chemical transport during neurodegeneration (Supported by ES-007148, ES-005022, and ES-015991).

PS 172 EFFECTS OF ESTROGEN-DERIVED COMPOUNDS IN A MURINE MODEL OF ALZHEIMER'S DISEASE.

A. E. Schlappal[1, 2, 3], R. Schuh[3, 4], L. Prokai[5] and M. Ottinger[1]. *[1]Animal and Avian Sciences, University of Maryland, College Park, MD, [2]Neuroscience and Cognitive Sciences Program, University of Maryland, College Park, MD, [3]Research and Development Service, Veterans Affairs Maryland Health Care System, Baltimore, MD, [4]Department of Neurology, University of Maryland School of Medicine, Baltimore, MD and [5]Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX.*

Studies have shown that estrogens have neuroprotective actions. Thus estrogenic compounds have become candidates for therapeutic interventions for reducing the incidence of developing Alzheimer's disease (AD) in postmenopausal women. However, the beneficial effects of estrogens are offset by the risk of increased incidence of estrogen sensitive-malignancies or venous thrombosis. Estrogen-derived para-quinol compounds have potential in providing neuroprotective benefits without having negative peripheral effects. We tested the efficacy of these estrogenic compounds in a transgenic mouse model (JAX Labs) that over-expresses human mutant forms of amyloid precursor protein (APP) and presenilin 1 (PS1). Female APP/PS1 transgenic mice and non-transgenic litter mates (6-8 months) were constantly exposed to vehicle (propylene glycol), estradiol (E2; 2ug/day), estrone (E1; 2ug/day), E2-quinol (0.2, 0.6, or 2ug/day), or E1-quinol (0.2, 0.6, or 2ug/day) by Alzet minipump (0.25ul/min) for 8 weeks. Mice were then assessed for cognitive function using a two-day radial-arm water maze (RAWM). Additionally, circulating E2 levels (serum) were measured by EIA. Our data show that APP/PS1 mice exposed to E2-quinol had fewer errors locating the hidden platform in the RAWM compared to APP/PS1 vehicle-treated mice, suggesting beneficial effect on cognitive function. These results provide supporting data for positive effects of E2-quinol on cognitive function in a transgenic model for Alzheimer's disease and suggest that this approach may hold promise for eventual biomedical applications.

PS 173 THE INTERACTION OF MANGANESE WITH PRION PROTEIN AND ITS ROLE IN THE PROPAGATION OF PRION DISEASE.

D. P. Martin[1, 2], V. Anantharam[1, 2], A. Kanthasamy[1, 2] and A. G. Kanthasamy[1, 2]. *[1]Department of Biomedical Sciences, Iowa State University, Ames, IA and [2]Iowa Center for Advanced Neurotoxicology, Iowa State University, Ames, IA.*

Protein misfolding and aggregation are considered key features of many neurodegenerative diseases, but biochemical mechanisms underlying protein misfolding and the propagation of protein aggregates are not well understood. Prion disease is a classical neurodegenerative disorder resulting from ectopic misfolding folding of endogenously expressed normal cellular prion protein (PrPC). Although the exact function of PrPC has not been fully elucidated, studies have suggested that it can

function as a metal binding protein. Interestingly, increased brain manganese (Mn) levels have been reported in various prion diseases indicating divalent metals may play a role in the disease process. Recently, our group found that Mn upregulated cellular prion protein levels in neuronal cell culture independent of transcription by significantly altering the turnover and stability of PrPC. To further understand the role of Mn in prion diseases, we examined the prion protein levels in animal models of Mn neurotoxicity. Administration of 10mg/kg of Mn once per day for 10-30 days by oral gavage induced regionally specific increases in PrPC levels, indicating that Mn can influence the level of prion protein in the brain. We also examined the effect of Mn on infectious cell culture models of prion disease. Treatment with 300μM Mn in persistently infected CAD5 cells showed reduced mitochondrial impairment, cytotoxicity, and caspase-3 activation when compared to uninfected cells. Scrapie infected cells also showed significantly reduced Mn uptake as measured by inductively coupled plasma-mass spectrometry (ICP-MS). Together, our data indicate that Mn interacts with prion protein to alter the stability of the protein and suggest that understanding the interaction of metals with disease specific proteins may provide further insights to prion-like propagation of neurodegenerative diseases. (Supported by NIH grant RO1ES19267 and MHRP grant W81XWH-05-10239)

PS 174 CHRONIC NEUROTOXICITY AFTER CHRONIC MOLD EXPOSURE.

R. M. Singer. *Raymond Singer, Ph.D., Professional Association, Santa Fe, NM.*

A 56 year old female with possible mold neurotoxicity was evaluated on 5 occasions from 2003-2008, after having resided in a contaminated, high-end condominium for 1.5 years. The evaluation included diagnostic interviews, neuropsychological tests, and medical/environmental record review. Shortly after moving into the contaminated residence, she had developed flu-like symptoms with nasal congestion, burning eyes, and fatigue. Environmental testing found mold including stachybotrys (SB) (3,424 counts of fungal structure (FS); 91,307 FS/m3, 97% of sample, categorized as massive fungal growth, with black mold in many locations in the apartment, including air samples of 260 particulates SB/M3. SB IgE was 51 ELISA units, above the reference range of 50. Her full scale IQ prior to exposure was estimated to be at the 97 percentile (pct). Post-exposure, she was unemployed since 2003 due to various cognitive malfunctions. MRI showed right temporal lobe abnormality. Results of the Neurotoxicity Screening Survey were elevated (436) and consistent with neurotoxicity, with Symptom Distortion not indicated. Current WAIS-III Working Memory was at the 5pct; WMS-III Working Memory 13pct; Selective Reminding Test 11 pct; Comprehensive Trail-Making Test 9pct; Controlled Oral Word Association Test 5pct, Stroop Color and Word Test 1 pct, indicating significant declines in memory and executive function. Repeated testing over 5 years after being removed from exposure found cognitive function partially improved, but her function did not return expected values.

PS 175 HYPERPHOSPHATEMIA AND HYPOZINCEMIA AFFECT PERIPHERAL NERVOUS SYSTEM IN SHR/NDMCR-CP, A MODEL OF METABOLIC SYNDROME.

Y. Suzuki[1], S. Ichihara[1], A. Kato[1], T. Yamaguchi[1], Y. Yamada[1] and G. Ichihara[2]. *[1]Life Science Research Center, Mie University, Tsu, Japan and [2]Nagoya University Graduate School of Medicine, Nagoya, Japan.*

Background: It is known that the patients with type 2 diabetes mellitus affect peripheral nervous system. However, it is unclear whether metabolic syndrome is associated with peripheral neuropathy. Given that patients with diabetic nephropathy are easily suffering from hyperphosphatemia and hypozincemia are clinically described, we assessed the higher phosphorus and lower zinc levels in plasma may affect peripheral nervous system using SHR/NDmcr-cp, a rat model of metabolic syndrome. We also investigated the effects of antioxidant, N-acetyl-L-cysteine (NAC), on peripheral nervous system under such conditions. Methods & Results: Male SHR/NDmcr-cp and control (WKY) rats were divided into 3 groups and were fed control diet (P 0.3% w/w, Zn 0.2% w/w) or a high-phosphorus and zinc-deficient (P 1.2 % w/w, Zn 0.0 % w/w) diet. The latter group was treated with either NAC (1.5 mg/g per day) or vehicle from 8 to 12 weeks of age (n = 6 or 8 for each group). Maximum motor nerve conduction velocity (MCV) and distal latency (DL) of the tail nerve were measured in rats at 12 weeks of age. The level of MCV was significantly smaller in CP control group than that of WKY control group. MCV of the tail nerve deteriorated more significantly by the high-phosphorus and zinc-deficient diet in CP compared with WKY. The treatment with NAC significantly prevented the toxic effects on the peripheral nervous system in both strains. Conclusion: Metabolic syndrome induces deterioration of MCV. Dietary high phosphorus and deficiency of zinc induces further damage to peripheral nerve system that may be protected by the treatment with antioxidant.

PS 176 EFFECTS OF 1-BROMOPROPANE EXPOSURE ON MYELINATION AND ASTROGLIA IN RAT BRAIN.

S. Sheik Mohideen[1], S. Ichihara[2], Z. Huang[1], L. Zhang[1], K. Subramanian[1], J. Kitoh[3] and G. Ichihara[1]. *[1]Department of Occupational & Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Mie University Graduate School of Regional Innovation Studies, Tsu, Mie, Japan and [3]Nagoya University, Nagoya, Japan.*

Introduction: 1-Bromopropoane (1BP) is used as an alternative to ozone depleting solvents. 1-BP exhibits neuro-reproductive toxicities in both animals and humans. A human case intoxicated with 1BP showed patchy areas of increased T2 signal in periventricular white matter in magnetic resonance image scan of the brain. We hypothesize that 1-BP might induce demyelination and affect myelination. The present study investigated the effects of 1-BP exposure on myelination through expression levels of genes and proteins related to myelination. Methods: Forty eight F344 rats were randomly divided into four groups of twelve each and exposed to 1BP at 0, 400, 800 and 1000 ppm for 8 hrs/day, 7 days/week for 4 weeks. Total RNA from brain parts was extracted and quantitative real time PCR was conducted to quantify the mRNA levels of Glial fibrillary acidic protein (Gfap), myelin basic protein (Mbp), OL lineage gene 2 (Olig2), myelin OL glycoprotein (Mog), chondroitin sulfate proteoglycan 4 (NG2), interleukin 11 receptor α (Il 11rα), caspase 3 (Casp3) and tumor necrosis factor α (Tnfα). 3 rats from each group was perfused using 4% paraformaldehyde for immunohistochemistry of Gfap, Mbp and oligodendrocyte marker O4. Results: In the cerebellum, mRNA levels of Mbp, Mog, Ng2, Tnfα and Olig2 genes decreased dose-dependently, while the mRNA level of Gfap gene increased dose-dependently. In the hippocampus, mRNA levels of the examined genes did not show any significant difference among groups. The Gfap immunostaining showed clearly higher expression in astrocyte including its glial process in the 1000ppm group when compared to the control. Conclusion: Thus exposure to 1-BP activated astroglia and decreased mRNA expression of myelin related genes in the rat cerebellum. 1BP exposure might disrupt myelination leading to astrocytes activation for CNS repair and remyelination.

PS 177 EXPOSURE TO 1-BROMOPROPANE DEGENERATES NORADRENERGIC AXONS IN THE RAT BRAIN.

G. Ichihara[1], S. Sheik Mohideen[1], S. Ichihara[2] and S. Nakamura[3]. *[1]Department of Occupational & Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Mie University, Tsu, Japan and [3]Neuroscience, Yamaguchi University Graduate School of Medicine, Ube, Japan.*

1-bromopropane (1-BP) has been used as an alternative to ozone-depleting solvents such as chlorofluorocarbons, which were used as cleaning agents for metal parts in electronics factories. Previous studies showed that 1-BP exhibits potent neurotoxicity. In humans, exposure to 1-BP caused a variety of neurological and neurobehavioral symptoms or signs including numbness and diminished vibration sense in the lower extremities and disturbance of memory. Moreover, depressed or irritated mood was reported by workers after exposure to 1-BP. However, neurobiological changes underlying depressive symptoms induced by exposure to 1-BP remain to be determined. Axonal degeneration of neurons containing noradrenaline (NA) and serotonin (5-HT) is thought to be associated with the occurrence of depressive symptoms. Based on this hypothesis, the present study examined whether repeated exposure to 1-BP causes the degeneration of 5-HT and NA axons. Exposure to 1-BP induced dose-dependent decreases in the density of NA axons in the rat brain, while the density of 5-HT axons did not appear to be affected by 1-BP. The present study suggests that depressive symptoms that occur after exposure to 1-BP are attributable, at least in part, to the degeneration of NA axons in the brain.

PS 178 CHRONIC EXPOSURE TO GLUCOCORTICOIDS PRIMES THE CNS PROINFLAMMATORY RESPONSE IN METHAMPHETAMINE NEUROTOXICITY.

K. A. Kelly, D. B. Miller and O. P. James. *Health Effects Laboratory Division, CDC-NIOSH, Morgantown, WV.*

Upregulation of proinflammatory cytokines and chemokines in the brain ("neuroinflammation") accompanies brain injury and disease as well as systemic infections. Previously we documented a neuroinflammatory response associated with the neurotoxic effects of the dopaminergic neurotoxicants, MPTP and METH. These elevations in a variety of proinflammatory mediators may serve as modulators or mediators of astroglial and microglial activation, cellular responses associated with all types of brain injury. Activated glia may have neuroprotective roles or may exacerbate neural damage. Our prior genetic and pharmacological interventions have resulted in partial suppression of neuroinflammatory responses associated with exposure to MPTP and METH without affecting neurotoxicity and gliosis. Because glucocorticoids are regarded as potent anti-inflammatory agents, we pretreated

mice with corticosterone (CORT) prior to administration of MPTP or METH and assessed a variety of cytokines/chemokines by qPCR and examined dopaminergic terminal damage and astrogliosis by tyrosine hydroxylase (TH) and GFAP immunoassay, respectively. Acute CORT (20 mg/kg, s.c.) 30 minutes prior to MPTP or METH reduced, but did not completely suppress the expression of LIF, CCL2, IL-1B induced neurotoxicity, whereas the decrease in TH and increase in GFAP remained unaffected. A chronic (1 week) CORT pretreatment in the drinking water was employed to achieve a longer and higher level of anti-inflammatory therapy on METH. Surprisingly, this CORT regimen appeared to prime the neuroinflammatory response to METH as most proinflammatory mediators showed exacerbated responses. In contrast to acute pretreatment with CORT, the effect of METH on TH and GFAP was exacerbated by chronic CORT. As the levels of chronic CORT approached or exceeded those associated with high physiological stress levels, our data suggest chronic CORT therapy or sustained physiological stress sensitizes CNS neuroinflammatory and neurotoxicity responses to METH.

PS 179 GST-PI INHIBITS DOPAMINE NEURON DEGENERATION IN *C. ELEGANS* MODELS OF PARKINSON'S DISEASE AND MANGANISM.

R. Settivari, J. LeVora, N. VanDuyn, S. Zhou, G. Sinclair and R. Nass. *Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.*

Idiopathic Parkinson's disease (PD) and manganism are oxidative stress-related movement disorders that result in abnormal dopamine (DA) signaling and cell death. Both neurological disorders involve basal ganglia and mitochondria dysfunction, and suggest overlapping epidemiology, yet the origin of the pathogenesis and the molecular determinants common between the diseases are ill-defined. Nrf2/SKN-1 regulates the gene expression of phase II detoxification enzymes, and is upregulated following exposure to oxidative stress in mammals and the nematode Caenorhabditis elegans (*C. elegans*). Glutathione-S-transferases (GSTs) of the class pi (GST-PI) are Phase II detoxification enzymes that conjugate both endogenous and exogenous compounds to glutathione to reduce cellular oxidative stress. GST-PI's have also recently been implicated PD-associated DA neurodegeneration. In this study we asked whether GST-PI expression may modulate DA neuron vulnerability following exposure to manganese (Mn) and rotenone. We demonstrate that a GST-PI homologue modulates DA neuron vulnerability to both of the PD-associated neurotoxicants. We show that SKN-1 and GST-PI are expressed in the *C. elegans* DA neurons, exposure to either toxicant increase reactive oxygen species, reduces mitochondrial membrane potential, induces GST-PI gene and protein expression, and the induction is partially dependent on SKN-1. We also show that GST-PI inhibits toxicant-induced DA neurodegeneration and toxicant-induced movement deficits, and identify cell death pathways involved in the neurodegeneration. Finally we present our initial studies from a reverse genetic screen that identifies modulators of PD-associated DA neuron vulnerability. Our studies provide the first *in vivo* linkage that a reduction in a xenobiotic metabolizing enzyme confers an increase in DA neuron vulnerability in models of PD and manganism. Support contributed by: NIH R011ES014459, NIH R01ES010563 (RN)

PS 180 ROLE OF GLIAL ACTIVATION IN A PROGRESSIVE NEUROINFLAMMATORY MODEL OF PARKINSON'S DISEASE USING MPTP AND PROBENECID.

B. R. Trout[1, 2], J. A. Miller[1, 2], K. A. Popichak[1] and R. B. Tjalkens[1, 2]. [1]*Environmental Radiological and Health Science, Colorado State University, Fort Collins, CO and* [2]*Molecular, Cellular and Integrative Neurosciences, Colorado State University, Fort Collins, CO.*

Inflammatory activation of glia is implicated in the progressive loss of dopaminergic neurons in Parkinson's Disease (PD). Suppression of neuroinflammation may prove useful in slowing the continued neuronal degeneration. In the present study we set out to investigate the efficacy of novel para-substituted diindolylmethane (cDIM) compounds in attenuating this progressive neuron loss. We first assessed cDIM activity in vitro using qPCR for NOS2, a prototypic neuroinflammatory gene. qPCR revealed attenuation of LPS induced NOS2 expression in astrocytes co-treated with cDIM5. Next, cDIM5 efficacy was assessed in vivo using the parkinsonian neurotoxicant, MPTP (2 x 15 mg/kg, bid 12 hr). Degeneration was determined by quantifying tyrosine hydroxylase loss in the striatum. Co-treatment of cDIM5 (50 mg/kg, daily oral gavage) returned striatal TH intensity to control levels. Lastly, we set out to establish a model of progressive neurodegeneration/neuroinflammation employing MPTP (80 mg/kg, total dose) in conjunction with probenecid (250 mg/kg). Mice were treated every other day for 7 days and monitored for a total of 14 days. To assess loss of dopaminergic neurons, stereological counts of TH positive neurons in the SNpc were determined on day 7 and day 14. Stereological counts revealed a reduction in the total number of dopaminergic neurons on day 7 which progressed to an even greater loss on day 14. These data show that upon cessation of MPTP/probenecid treatment, loss of nigral neurons continues to occur, emulating a progressive lesion as seen in PD.

PS 181 DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING PLATFORM FOR MONOAMINE TOXICITY.

A. I. Bernstein[1], K. A. Stout[1], J. D. de Gastyne[1] and G. W. Miller[1, 2]. [1]*Environmental Health, Emory University, Atlanta, GA and* [2]*Center for Neurodegenerative Disease, Emory University, Atlanta, GA.*

The vesicular monoamine transporter 2 (VMAT2) is responsible for packaging monoamine neurotransmitters such as dopamine (DA), serotonin, norepinephrine and epinephrine into synaptic vesicles. Levels of these monoamines are highly regulated and their dysregulation plays a role in Parkinson's disease, Huntington's disease, drug addiction, neuropsychiatric disorders and pesticide toxicity. While the primary function of VMAT2 is to sequester these monoamines into vesicles, they can also translocate toxicants away from cytosolic sites of action. Identification of compounds that modify the action of VMAT2 may be useful as possible therapeutic agents for preventing or reversing monoamine-related toxicity. To this end, we are utilizing the fluorescent substrate, 4-(4-dimethylaminostryl)-N-methylpyridinium (ASP+) to develop a high throughput screening assay. ASP+ is transported into cells by the plasma membrane transporters of DA, norepinephrine and serotonin and has been developed into a rapid, high-throughput assay for function of these transporters (Molecular Devices). We demonstrate here that ASP+ is also transported in vesicles by VMAT2 and uptake can be blocked by the VMAT2-specific inhibitor tetrabenazine (TBZ). HEK cell lines stably expressing the dopamine transporter and VMAT2 were generated. Uptake of 3H-DA was measured in parallel to uptake of ASP+. Time course experiments indicated a similar time course of uptake with maximal signal occurring at 20 minutes. Dose response experiments with TBZ demonstrated that uptake of 3H-DA and ASP+ is inhibited by similar concentrations of TBZ. We are currently optimizing this assay into a platform amenable for high-throughput screening for VMAT2 function. Supported by NIEHS P01ES016731.

PS 182 SEROTONERGIC AND NORADRENERGIC SYSTEMS ARE DISRUPTED IN THE VMAT2-DEFICIENT PARKINSON'S DISEASE MOUSE MODEL.

S. P. Alter, T. N. Taylor, K. R. Shepherd and G. W. Miller. *Environmental Health, Emory University, Atlanta, GA.*

Our lab has established that mice with reduced vesicular monoamine transporter 2 (VMAT2; Slc18a2) display motor and nonmotor symptoms of Parkinson's disease (PD). Previously, we demonstrated dopaminergic degeneration occurs in VMAT2-deficient mice, paralleling the pathology of human PD (J Neuroscience, 2007; 2009). Here, we report disruptions in the noradrenergic and serotonergic systems in the VMAT-2 deficient mouse model. It is known that the locus ceruleus (LC) undergoes degeneration prior to clinical presentation of motor symptoms in human PD. Similarly, stereological analysis revealed progressive LC degeneration in the VMAT2-deficient mouse model, beginning at 12 months of age. At 18 months, this degeneration is more drastic than that of the substantia nigra. Consistent with the notion that oxidative damage can drive neurodegeneration, DCF assays show that primary cultured LC neurons from VMAT2-deficient mice are sensitive to oxidative challenge when treated with dopamine and norepinephrine. WT LC neurons are protected from this effect. Serotonergic dysfunction also occurs in PD. Previous work by our lab has shown that VMAT2-deficient mice brains have decreased levels of serotonin and decreased serotonin turnover. Here, we show that VMAT2 loss adversely affects the dorsal raphe, leading to markedly increased cell death as detected by silver staining in 24 month old VMAT-2 deficient mice, in contrast to age-matched WT, and we observed an increase in alpha-synuclein accumulation in 30 month old mice. DCF assays revealed a 60 % increase in serotonergic-induced oxidative stress in cultured postnatal VMAT2-deficient raphe neurons. In tail-suspension tests, VMAT2-deficient mice display greater immobility time than age-matched wildtype, and this is attenuated with fluoxetine treatment. These findings show that the VMAT2-deficient mice experience serotonergic and noradrenergic pathologies that are similar to those in human PD. Supported by 5P01ESO16731.

PS 183 VASCULAR IMPLICATIONS OF NRTI-INDUCED MITOCHONDRIAL TOXICITY.

V. Y. Hebert, S. Xue, M. Glover and T. Dugas. *Pharmacology, Toxicology, and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, LA.*

Associations between the use of nucleoside analog reverse transcriptase inhibitors (NRTIs) in the treatment of HIV and various cardiovascular side effects have been well-documented; however, the precise mechanisms underlying NRTI-induced cardiovascular pathogenesis have not yet been fully elucidated. Previous work from our

laboratory has demonstrated that NRTI treatment in vivo induces direct endothelial dysfunction, a hallmark of atherogenesis, while results from in vitro studies have suggested that compromised mitochondria may play a role in NRTI-induced endothelial dysfunction. In the present study, human umbilical vein endothelial cells (HUVEC) treated with micromolar concentrations of the NRTIs zidovudine, lamivudine, and tenofovir disoproxil fumarate were found to have increased levels of reactive oxygen species (ROS), decreased activity of mitochondrial complexes I, II+III, and IV, decreased ATP production, and a decreased NAD+/NADH ratio as early as 4-6 hrs after treatment. Moreover, levels of two markers for endothelial dysfunction and activation, vascular cell adhesion molecule-1 and the vasoconstricting peptide endothelin-1, were increased after NRTI treatment. Interestingly, ROS levels and ATP production return to levels comparable to those in untreated cells after 18-24 hrs of treatment, suggesting the existence of a compensatory mechanism for the clearance of damaged mitochondria. Using confocal microscopy, we have shown specific colocalization of mitochondria with lysosomes and early autophagosomes in treated cells, which is indicative of mitochondrial autophagy (mitophagy), a phenomenon which may be involved in the removal of NRTI-damaged mitochondria. Thus, short-term endothelial NRTI toxicity may manifest in a compensated state, whereas more chronic exposure may result in pathogenic maladaptation. These data taken together with our prior studies may suggest a potentially causal relationship between NRTI-induced disruption of mitochondrial function and endothelial dysfunction, as well as a distinction between short-term and long-term effects of NRTI treatment.

PS 184 DRUG-INDUCED CARDIAC LEFT VENTRICULAR DYSFUNCTION AND ARRHYTHMIA ASSOCIATED WITH MITOCHONDRIAL IMPAIRMENT.

P. J. Acton, K. Derakhchan, R. W. Chui, Y. Qu, M. Fang, B. Gao, I. Senal, R. Hernandez, D. Robert, H. M. Vargas and J. W. Lawrence. *Comparative Biology and Safety Sciences, Amgen, Thousand Oaks, CA.*

Early stage safety assessment assays are used to profile agents for their potential to cause cardiac electrical and mechanical (contractility) dysfunction. During development of a drug candidate series, left ventricular contractility deficits were identified as a common finding in the chloralose-anesthetized dog model. In one case, a candidate molecule also evoked polymorphic ventricular tachycardia. Investigations of off-target mechanisms demonstrated that cardiac β1-adrenoceptor and sodium/calcium channel blockade could not account for the contractility deficit. A custom built mitochondrial function profiling platform was used to assess potential mitochondrial mechanisms associated with reduced left ventricular contractility. Profiling mitochondrial mechanism demonstrated that the left ventricular contractility deficits were associated with potent mitochondrial complex V (ATP synthase) inhibition and did not involve electron transport chain inhibition, TCA or fatty acid substrate oxidation inhibition, mitochondrial membrane potential, mitochondrial permeability transition, or calcium loading potential deficiencies. The concentrations that inhibited complex V were also shown to inhibit contractility in the isolated rabbit heart preparation. Assessment of several structurally similar compounds clearly demonstrated that the contractility decreases were associated with complex V inhibition and unrelated to sodium and calcium channel mechanisms. We conclude that these agents reduced left ventricular contractility through inhibition of mitochondrial ATP synthase. In addition, ATP synthase inhibition in concert with hERG blockade may be pro-arrhythmic.

PS 185 INTERFERON SIGNALING, SYSTEMIC INFLAMMATION, AND ATHEROSCLEROSIS FOLLOWING WELDING FUME INHALATION EXPOSURE: FROM THE LUNG TO THE BLOOD TO THE VASCULATURE.

A. Erdely, T. Hulderman, A. L. Liston, R. Salmen-Muniz, S. Stone, B. T. Chen, D. G. Frazer, S. Li, M. L. Kashon, J. M. Antonini, P. P. Simeonova and P. C. Zeidler-Erdely. *NIOSH, Morgantown, WV.*

Inhalation of particulates can result in measurable systemic markers related to adverse cardiovascular outcomes. Our aim was the determination of a systemic signature and atherosclerosis progression following gas metal arc-stainless steel (GMA-SS) welding fume exposure. C57BL/6 mice were exposed for 10d to GMA-SS by inhalation (40mg/m3 for 3hr/day) and harvested 4hr, 14d and 28d post-exposure. Systemic responses were measured by serum protein profiling and microarray (Ingenuity pathway analysis and real-time RT-PCR confirmation) for whole blood cells, aorta and lung. Atherosclerotic susceptible apolipoprotein E knockout (apoE-/-) mice were exposed (40mg/m3 for 3hr/day) during week 6 and 7 of two months of Western (high fat) diet feeding. Exposed C57BL/6 mice exhibited a specific network of type I interferon signaling in blood cells and aorta from 4hr to 28d post-exposure. The central component of the network was the transcription factor interferon regulatory factor 7 (Irf7), the master regulator of the type I interferon response. Increases in related genes (Oasl2 and Ifitm3) were also validated. Type I interferon signaling was also a top network in the lung supporting the concept of a systemic signature reflecting the ongoing pulmonary response. Serum levels of primary inflammatory mediators were not elevated although oncostatin M, an IL-6 family cytokine and type I interferon enhancer, was increased. In apoE-/- mice, pulmonary inflammation, serum levels of IL-1β and MAC-3 and blood cell expression of Irf7 were increased post-GMA-SS exposure. Atherosclerosis severity, determined by plaque area and intensity in the aorta (en face), was increased. The data suggest an immunomodulatory and mild systemic inflammatory effect with increased progression of atherosclerosis due to GMA-SS exposure. Most notably, a pulmonary and corresponding systemic signature of type I interferon signaling was consistently evident across tissue and time.

PS 186 A COMMON LINK FOR PULMONARY INFLAMMATION TO VASCULAR EFFECTS OF ENVIRONMENTAL TOBACCO SMOKE AND BENZO-A-PYRENE IN BOTH PIGS AND RATS.

L. P. Weber, A. N. Al-Dissi and N. J. Gentner. *Toxicology Graduate Program, University of Saskatchewan, Saskatoon, SK, Canada.*

Despite extensive research, the mechanisms behind cardiovascular effects of prolonged environmental tobacco smoke (ETS) remain unclear, but may be related to ETS-induced inflammation, oxidative stress and endothelial dysfunction. We hypothesized that polycyclic aromatic hydrocarbons (PAHs) in ETS are responsible. Parallel studies were carried out in rats and juvenile pigs to examine the cross-species consistency of the role of cytochrome P450 1A1 (CYP1A1), inflammation and oxidative stress in causing adverse cardiovascular effects. Male juvenile pigs were exposed to ETS or ambient air for 28 days (1 hr/day) and effects compared to 7 days of intravenous injection of the PAH, benzo-a-pyrene (BaP; 5 mg/kg). The pig experiments were compared to a similar 28-day ETS exposure or 7-day intranasal BaP (0.01 mg/kg) instillation in male Sprague-Dawley rats. As early as 7 days in both species, ETS caused impaired endothelial function (indicated by impaired flow-mediated dilation or FMD) in pigs, while causing increased arterial stiffness (indicated by increased arterial pulsewave dP/dt) in rats. Intranasal BaP mimicked the ETS effects to cause increased arterial stiffness in rats, but intravenous BaP in pigs did not affect FMD. Both ETS and BaP caused a significant increase in serum nitrotyrosine (both species) and C-reactive peptide (pigs), but had inconsistent effects on indicators of total NO production. ETS exposure resulted in increased CYP1A1 activity in the lung but not the liver in both species, while BaP treatment had no effect (rats) or the opposite effect in both organs (pigs). However, there was a consistent increase in pulmonary white blood cells (WBCs) after ETS exposure (both species) and after intranasal BaP (rats), but not after intravenous BaP (pigs). In conclusion, increased pulmonary WBCs after ETS or intranasal BaP exposure may be the common link to increased oxidative stress and endothelial dysfunction in multiple animal models, suggesting these findings may be broadly applicable to all species.

PS 187 CIGARETTE SMOKE-DEPENDENT CHANGES IN THE RESPIRATORY AND CARDIOVASCULAR SYSTEM OF SPONTANEOUSLY HYPERTENSIVE (SH) RATS AND THE EFFECT OF SMOKING CESSATION.

M. Lietz[1], B. Friedrichs[1], I. Gallitz[1], S. Quadt-Humme[1], Y. Steffen[1], K. Stolle[1], S. Lebrun[2] and A. Berges[3]. [1]*Philip Morris Research Laboratories GmbH, Cologne, Germany,* [2]*Philip Morris Products S.A., Neuchatel, Switzerland and* [3]*Philip Morris Research Laboratories Bvba, Leuven, Belgium.* Sponsor: T. Mueller.

Previously, we have shown that cigarette smoke affects cardiac function, hypertrophy status, and serum proteins in SH rats (Abstract 912; 48th Annual Meeting & ToxExpoTM [2009]). Here, we investigate the reversibility of these effects after smoking cessation (SC). SH rats were exposed to diluted cigarette mainstream smoke (MS) from the Reference Cigarette 3R4F (MS-only; 900 μg total particulate matter/day; 5 days/week) or to fresh air (sham) for 90 days. SC experiments covered exposure for 30 days MS + 60 days sham (SC-1) and 60 days MS + 30 days sham (SC-2). Endpoints tested were body weight, heart hypertrophy status, neutrophils in bronchioalveolar lavage fluid, expression levels of matrix metalloproteinase-1 (MMP-1) and metallopeptidase inhibitor-1 (TIMP-1). At 90 days, parameters were decreased (body weight) or increased (all other endpoints) in the MS-only group compared to both cessations groups, which, importantly, display a sham-like phenotype: Body weight [g]: 275.9+16.8 (MS-only), 316.0±18.5 (SC-1), 311.8+19.6 (SC-2), 301.7±28.7 (Sham); Hypertrophy status [left ventricle weight (g)/tibia length (mm)]: 0.023±0.002 (MS-only), 0.020±0.001 (SC-1),

0.021±0.001 (SC-2), 0.019±0.002 (Sham); TIMP-1 [pg/ml]: 1990.0±219.6 (MS-only), 247.0±12.4 (SC-1), 234.7±11.5 (SC-2), 270.6±19.7 (Sham); MMP-1 [ng/ml]: 7.3±0.4 (MS-only), 3.2±0.4 (SC-1), 3.0±0.4 (SC-2), 3.8±0.6 (Sham); Neutrophil count [%]:12.7±1.1 (MS-only), 0.7±0.1 (SC-1), 3.6±0.6 (SC-2), 0.4±0.1 (Sham). Further endpoints are currently under investigation. Hence, this experimental format may turn out to be suitable to evaluate respiratory and cardiovascular effects of potentially reduced-risk tobacco products.

PS 188 INHALATION OF ENVIRONMENTALLY PERSISTENT FREE RADICALS ALTER LEFT VENTRICULAR FUNCTION *IN VIVO* .

S. Mahne[1], G. Raman[1], B. Dellinger[2] and K. J. Varner[1]. [1]*Pharmacology, Louisiana State University Health Sciences Center, New Orleans, LA and* [2]*Chemistry, Louisiana State University A&M, Baton Rouge, LA.* Sponsor: T. Dugas.

Epidemiological data consistently link exposure to increased fine airborne particulate matter with increased cardiovascular morbidity and mortality. We have shown that during combustion, halogenated hydrocarbons chemisorb to the surface of transition metal-oxide-containing particles and form an environmentally persistent free radical (EPFR) capable of continuous redox cycling. The purpose of this study was to determine whether inhalation of EPFRs adversely affects baseline cardiac function in naïve rats. To test the physiological effects of EPFRs we developed a surrogate particle system, dichlorobenzene-230 (DCB230). Rats were exposed to either vehicle or DCB230 via nose only inhalation for 20 min/day for 7 days. 24 hrs after the final exposure, the rats were anesthetized, intubated and left ventricular function was measured using a Millar pressure-volume catheter. Exposure to DCB230 (~600μg/m3) increased systolic function as evidenced by augmented dP/dt, stroke work (SW), ejection fraction and cardiac output (CO). This dose also increased diastolic function as evidenced by decreases in the end diastolic pressure and an increase in the relaxation constant Tau W. After exposure to DCB230 (~1000μg/m3) diastolic function was further enhanced. However, end diastolic volume, stroke volume and CO were significantly decreased, while measures of contractility (dP/dt, end systolic pressure and SW) were at control level. These data indicate that the higher conc. of DCB230 reduced ventricular filling which may reflect an increase in pulmonary vascular resistance due to lung inflammation. The mechanism underlying the increased function produced by the lower DCB230 conc. is less clear but may reflect sympathetic stimulation due to a milder pulmonary inflammation. These data show that inhalational exposure to EPFR can significantly affect cardiac function in vivo and that pulmonary inflammation may play a contributory role. Support P20ES013648, P20RR18766.

PS 189 EFFECTS OF INHALED NITRIC OXIDE AND CARBON MONOXIDE ON VASCULAR RESPONSIVENESS.

J. G. Buntz, S. N. Lucas and M. Campen. *Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Cardiovascular disease (CVD) remains a major cause of morbidity and mortality in the developed and developing world. Exposure to emissions from diesel and gasoline engines is becoming widely recognized as an important risk factor for CVD. Previously, we have shown the negative effects of whole diesel emissions on systemic arteries in rats. In an effort to determine the drivers of toxicity in the complex diesel exhaust mixture, we have begun investigating the effects of nitric oxide (NO) and carbon monoxide (CO), two of the primary components of diesel emissions, on vascular function in systemic arteries. Whole body rodent exposures were conducted for both NO and CO or room air for one or four hours. CO exposure was 0, 30 and 100 ppm; NO exposure levels were 0 and 10 ppm. Immediately after exposures, animals were euthanized for tissue collection. Aortic rings, two per animal, were mounted on a force-transduction myograph (Danish Myo Technology) and challenged with potassium, phenylepherine (PE) and acetylcholine (ACh). Additionally, plasma nitrate/nitrite levels were measured to assess the effect of the NO exposures. We found that acute CO exposures altered dilatory responses to ACh in rat carotid arteries in a biphasic manner, with the low exposure potentially improving dilation compared to controls (n.s.) and the high concentration impairing function ($p<0.05$). Acute NO exposures did not affect either the dilation response to ACh or constriction response to PE in mouse aortas. Interestingly, supplementation with N-acetylcysteine (NAC), given by intraperitoneal injection (200 μM/kg) prior to NO exposures led to enhanced dilation (n.s.), suggesting an interactive effect of inhaled NO and NAC in the dilatory response to ACh. Plasma nitrate/nitrite levels were significantly elevated by inhalational exposure to NO, with a slight but nonsiginificant increase by NAC supplementation. These results suggest that ambient levels of CO may have an important contribution to vascular function, while NO alone in a healthy animal model had minimal impact.

PS 190 EXPOSURE TO ACROLEIN ENHANCES PLATELET ACTIVATION AND EXACERBATES ATHEROSCLEROSIS.

S. Srivastava, S. D. Sithu, E. N. Vladykovskaya, P. Haberzettl, D. J. Hoetker, M. A. Siddiqui, D. J. Conklin, A. Bhatnagar and S. E. D'Souza. *School of Medicine, University of Louisville, Louisville, KY.*

Acrolein is a ubiquitous pollutant that is abundant in automobile exhaust, tobacco smoke and industrial waste and emissions. Acrolein is also present in high concentrations in alcoholic beverages and foods including cheese, donuts and coffee. Exposure to acrolein containing environmental pollutants such as tobacco smoke and automobile exhaust has been linked to platelet activation and vascular dysfunctions and thereby to the pre-disposition of athero-thrombotic events in human. In the present study we show that acute (5 ppm for 6 h) or sub-chronic (1 ppm, 6h/day for 4 days) acrolein inhalation exposures as well as oral exposure to acrolein (5mg/kg by gavage; 24h) in male C57BL/6 mice causes platelet activation as evident by increased ADP-induced platelet aggregation (20%), increased platelet-leukocyte aggregates in the blood (1.6-2.0 fold), enhanced plasma PF4 levels (2.5 fold), and increased platelet-fibrinogen binding (1.5 fold). The bleeding time in acrolein-exposed mice was decreased by 40%. Furthermore, oral exposure to acrolein (2.5 mg/kg/day by gavage for 8 weeks) in 8 week old male apoE-null mice, increased the concentration of cholesterol in the plasma by 20% and the abundance of small and medium VLDL particles by 25-30%. Acrolein feeding also increased atherosclerotic lesion formation in the aortic valve by 2-fold and augmented the accumulation of macrophages in the lesions. Accumulation of PF4 in the plasma and in atherosclerotic lesions was also significantly increased in acrolein-fed mice. Incubation of endothelial cells with the plasma of acrolein-fed mice enhanced transmigration of monocytic cells by 2-fold, which was abolished by anti-PF4 antibody treatment. Together, our data suggest that acrolein is an important contributing factor to the pro-thrombotic and pro-atherogenic risk in human exposure to pollutants such as tobacco smoke or automobile exhaust, or through dietary consumption.

PS 191 SOLUBLE COMPONENTS OF ULTRAFINE PARTICULATE MATTER ACTIVATE CORONARY ENDOTHELIAL CELLS.

S. J. Snow[1] and M. Carraway[2]. [1]*Curriculum of Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, NC and* [2]*Human Studies Facility, National Health and Environmental Effects Research Laboratory, U.S. EPA, Chapel Hill, NC.*

Exposure to particulate matter (PM) is associated with increased cardiovascular morbidity and mortality. Ultrafine (UF) particles are of particular importance in cardiovascular dysfunction due to air pollution. The mechanisms underlying these effects are not known, and we hypothesize that the soluble components of UF particles could leave the lung and interact with vascular cells. We tested the hypothesis that the soluble fraction of UF particles induce reactive oxygen species (ROS) production and activate pro-inflammatory and pro-coagulant changes in endothelial cells. We quantified extracellular H2O2 production using the Amplex Red assay in human coronary artery endothelial cells immediately following exposure to either UF particles, or their insoluble and soluble fractions. We determined that the soluble components of UF particles are primarily responsible for increased H2O2 production. Increased ROS production was confirmed using confocal microscopy and the fluorescent indicator Peroxy Green 1 (PG1), which is specific for intracellular H2O2. In addition, exposure to the soluble fraction of UF particles at 10, 50, and 100 μg/ml induced significant increases in mRNA expression of pro-inflammatory mediators IL-8, IL-1β, and E-selectin, and of pro-coagulant factors tissue plasminogen activator and tissue factor at both 6 and 24 hrs. Increased ROS production and enhanced mRNA expression of these key proteins indicate endothelial activation by the soluble components of UF PM. This change in endothelial phenotype is a plausible mechanism that contributes to accelerated atherosclerosis and other cardiovascular diseases associated with exposure to air pollution.

PS 192 THE EFFECTS OF CERIA NANOPARTICLES ON THE REACTIVITY OF RAT MESENTERY AND CORONARY ARTERIOLES.

V. C. Minarchick[1], E. M. Sabolsky[2] and T. R. Nurkiewicz[1]. [1]*Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown, WV and* [2]*Mechanical and Aerospace Engineering, West Virginia University, Morgantown, WV.*

Metal oxide nanoparticles have a variety of uses but research assessing their toxicity is limited. Previous work in our lab has shown pulmonary exposure to titanium dioxide (TiO_2) alters reactivity in coronary resistance arterioles. However, the microvascular effects of other metal oxides, such as ceria nanoparticles (CeO_2) is un-

known. The aim of this study was to analyze the effects of pulmonary CeO_2 exposure on mesenteric and coronary microvascular function. Sprague-Dawley rats were intratracheally instilled with CeO_2 (100 μg/rat). The CeO_2 used had a primary diameter of ~3 nm, which was determined via transmission electron microscopy. 24 hours post exposure, the rats were anesthetized with isoflourane and the heart and mesentery were removed. Arterioles (<150 μm) were isolated from the heart and mesentery. The vessels were suspended between glass micropipettes and attached with silk sutures. The vessels were superfused with physiological salt solution, and brought up to a set pressure (45 mmHg for coronary vessels and 80 mmHg for mesentery vessels) and temperature (37°C). Vessels were allowed to develop spontaneous tone before being assessed for endothelium-dependent [A23187 (10^{-9}-10^{-6} M) and acetylcholine (ACh, 10^{-9}-10^{-4} M)] and -independent reactivity [sodium nitroprusside (SNP, 10^{-9}-10^{-4} M)]. Responsiveness to phenylephrine (PE, 10^{-9}-10^{-4} M) and pressure (0-105 mmHg) was also assessed. Results indicate that CeO_2 exposure does not significantly alter myogenic responsiveness but appears to impair nitric oxide (NO) sensitivity. These preliminary results suggest that microvascular dysfunction follows ceria nanoparticle exposure, but does not appear to be identical to the dysfunction observed after exposure to other nanoparticles.
Support: NIH-RO1-ES015022 and RC1-ES018274 (TRN).

PS 193 EXPOSURE TO MULTI-WALLED CARBON NANOTUBES DOSE-DEPENDENTLY EXACERBATES CARDIAC INJURY AND DEPRESSES CORONARY FLOW.

L. C. Thompson[1], C. R. Frasier[1], R. C. Sloan[1], D. A. Brown[1], B. Harrison[3], J. M. Brown[2] and C. J. Wingard[1]. [1]*Physiology, East Carolina University, Greenville, NC,* [2]*Pharmacology and Toxicology, East Carolina University, Greenville, NC and* [3]*Wake Forest University Institute of Regenerative Medicine, Winston-Salem, NC.*

Multi-walled carbon nanotubes (MWCNT) have unique physicochemical properties that allow for numerous commercial applications; yet these properties may present a potential risk to physiological systems. We hypothesized that pulmonary instillation of MWCNTs would increase cardiac ischemia/reperfusion (I/R) injury. Male Sprague-Dawley rats were instilled with 1, 10, or 100 μg of commercial grade (C-grade) MWCNT in 200 μl of 10% surfactant/saline (10%SS) or a 10% SS vehicle. 24 hours post-instillation hearts were mounted on a modified Langendorff apparatus and subject to 20/120 minute global ischemia/ reperfusion. MWCNT instillation resulted in significant dose-dependent infarct expansion at 100 μg (68.3 ± 7.6 % of the zone at risk), as compared to the vehicle (52.9 ± 4.1 %). Coronary flow rates during reperfusion were dose-dependently attenuated with significance at 100 μg (4.9 ± 0.6 ml/min/g) compared to vehicle (6.8 ± 0.6 ml/min/g). Segments of the left anterior descending (LAD) coronary artery were isolated from rats exposed to vehicle or 100 μg MWCNT and mounted on wire myograph. There was increased active force production during vasoconstriction responses to 0.1 – 1.0 μM ET-1 from 100 μg vessels (3.7 ± 0.2 mN/mm2) as compared to vehicle (2.2 ± 0.2 mN/mm2). The responses to 5-HT showed a similar elevation in active force over the 10.0 μM – 100.0 μM dose range from 100 μg (3.4 ± 0.1 mN/mm2) compared to vehicle (0.7 ± 0.1 mN/mm2). We observed no differences in the relaxation responses to SNP or Ach. These data suggest that an acute pulmonary exposure to MWCNTs can drive conditions favoring cardiac I/R injury by elevated coronary vasoconstriction. This work is supported by NIH RO1 ES019311(JMB)and ES016246(CJW).

PS 194 TIME-COURSE OF IMPAIRED CORONARY ARTERIOLAR ENDOTHELIUM-DEPENDENT DILATION AFTER MULTI-WALLED CARBON NANOTUBE INHALATION.

P. G. Stapleton[1, 3], V. C. Minarchick[1, 3], A. Cumpston[2], W. McKinney[2], B. T. Chen[2], D. Frazer[2], V. Castranova[2] and T. R. Nurkiewicz[1, 3]. [1]*Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown, WV,* [2]*Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV and* [3]*Physiology and Pharmacology, West Virginia University, Morgantown, WV.*

We have shown that exposure to fine particulate matter (PM), and more recently, nanoparticules, can lead to changes in reactivity within the skeletal muscle and coronary microvasculature. Multi-walled carbon nanotubes (MWCNT) are becoming widely used in many fields to enhance existing materials for strength, conduction, and isolation. Rats were exposed to filtered air (control) or MWCNT with count mode aerodynamic diameter of 420 nm via a single day 5-hour inhalation exposure (+/- 20 min) at concentrations of 26 ± 2 mg/m^3 which produced calculated pulmonary depositions of 22 ± 2 μg. Coronary arterioles were isolated from the left anterior descending (LAD) artery distribution and isolated microvessel analysis of sub-epicardial arterioles (~150 μm in diameter) was performed based on responses to transmural pressure and to increasing concentrations of phenylephrine (PE), acetylcholine (ACH), the Ca^{2+} ionophore A23187, and sodium nitroprusside (SNP) over a time course of 24, 72, 120, or 168 hours post MWCNT exposure. There were no significant differences observed in the endothelium-independent responses to SNP and PE indicating smooth muscle sensitivity to nitric oxide (NO) and adrenergic responses were intact. Vascular reactivity to increasing concentrations of ACH lead to significant differences from controls for each of the points within the post-exposure time course (24, 72, 120, and 168 hours) indicating that: 1) MWCNT inhalation impairs endothelium-dependent dilation and 2) this systemic microvascular effect lasts for at least one week post exposure. These findings are consistent with other nanoparticle outcomes in addition to the cardiac events described after PM exposure.
Support: NIH-RO1-ES015022 and RC1-ES018274 (TRN).

PS 195 PROLONGATION OF QT INTERVALS BY MOXIFLOXACIN TREATMENT IN 2 AND 8 WEEK OLD JUVENILE BEAGLE DOGS.

M. Peschar[1], N. Lourens[1], K. Scase[1], A. Zmarowski[1], M. De Raaf[1], E. Padgett[2], B. Van Rozendaal[1] and H. Emmen[1]. [1]*Toxicology, NOTOX B.V., DD's-Hertogenbosch, Netherlands and* [2]*Toxicology, WIL Research Laboratories LLC, Ashland, OH.*

Many drugs used in juveniles lack clinical data obtained from pediatric populations, and several ethical and technical considerations are inherent to pediatric clinical trials making them difficult to conduct. Thus, safety testing in juvenile animals is critical to determine potentially adverse effects. Delayed ventricular re-polarization measured by QT interval length is important in pre-clinical studies as QT interval prolongation may indicate a risk for potentially lethal ventricular arrhythmia. Little pre-clinical data is available on QT prolongation in juveniles. As such, a juvenile study was conducted to assess the feasibility of measuring and analyzing ECGs in Beagle dogs as young as 2 and 8 weeks of age. In this study 10 mg/kg Moxifloxacin (a positive control that prolongs QT intervals) and 0.5% methylcellulose (vehicle) were administered to 2 male and 2 female dogs at 2 and 8 weeks. ECGs were recorded at pre-dose, at 1, 2, 3, 4 and 24 hours post-dose, and QT intervals were determined. The vehicle was administered first with Moxifloxacin one day later. Valid ECG-data could be collected at all time points. Moxifloxacin prolonged the absolute QT intervals at post-dose measurements at both ages (all animals). The QT interval was longer at 8 weeks than at 2, which may parallel normal development of animals at these ages; the Moxifloxacin-induced increase was also more pronounced over this period. QT intervals were not significantly longer than pre-dose measurements at either age after vehicle administration. Further, since QT intervals vary with heart rate, suitable correction factors were also analyzed; the individual Sarma QT correction was the most accurate correction factor. This study was the first to measure ECGs and induce QT prolongation in dogs as young as 2 and 8 weeks using Moxifloxacin. This approach may ultimately help screen pharmaceuticals for adverse effects on ventricular re-polarization in pre-clinical juvenile populations.

PS 196 REGULATION OF CARDIAC CARDIOTROPHIN-1 GENE EXPRESSION BY VALSARTAN AND SPIRONOLACTONE IN RAT, A POSSIBLE MECHANISM OF CARDIOPROTECTION.

H. A. Al-Mazroua, N. M. Al-Rasheed and H. M. Korashy. *Pharmacology & Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.*
Sponsor: A. El-Kadi.

Heart failure (HF) is an epidemic public health problem in the globe. The incidence, prevalence, and hospitalization rates associated with HF are projected to increase substantially, as it is approximately estimated to affect more than one fifth of the Saudi population. Among all medications used clinically to treat HF, spironolactone (SPL) and valsartan (VAL), have been shown to reduce morbidity and mortality in patients with HF. In the context that a novel cardiac gene Cardiotrophin 1 (CT-1) has been recently shown to play a crucial role in the pathogenesis of HF, this study was undertaken to investigate weather VAL and SPL exert cardioprotective effect through the modulation of *CT-1* gene expression. For this purpose, 36 adult male Wistar Albino rats were allocated into six groups as follows; control rats, isopreterenol (ISO)-induced HF rats, VAL-treated rats, SPL-treated rats, ISO+VAL-treated rats, and ISO+SPL-treated rats. Plasma CT-1 levels were measured by enzyme-linked immunosorbent assay (ELISA). Thereafter, heart tissues were dissected and the heart to body weight ratio were determined. CT-1 mRNA and protein expression levels were determined by Real-Time Polymerase Chain Reaction (RT-PCR) and Western blot analysis, respectively. Our results showed that treatments of rats with ISO significantly induced HF as indicated by elevated heart/body weight

ratio, increased expression of cardiac hypertrophic genes. This was accompanied by increased CT-1 plasma levels as compared to control rats. Although administration of VAL or SPL alone did not significantly alter *CT-1* gene expression, co-treatment of ISO with either VAL or SPL dramatically decreased plasma levels of CT-1 and inhibited its mRNA and protein expression levels. In conclusion, this work provides the first evidence that VAL and SPL inhibited *CT-1* gene expression at the transcriptional and posttranscriptional levels and may suggest a novel mechanism of cardioprotective effect of VAL and SPL.

PS 197 TCDD MISREGULATES CELL MIGRATION AND THE EXTRACELLULAR MATRIX IN THE REGENERATING ZEBRAFISH HEART.

P. J. Hofsteen[1], M. Kim[1], D. Nesbit[1], V. Mehta[2], R. E. Peterson[1, 2] and W. Heideman[1, 2]. [1]*School of Pharmacy, University of Wisconsin Madison, Madison, WI and* [2]*Molecular and Environmental Toxicology, University of Wisconsin, Madison, WI.*

Mammalian hearts lack the ability to regenerate after wounding whereas zebrafish (Danio rerio) hearts regenerate after surgical removal of ~20% of the ventricle apex. Treatment of adult zebrafish (AB) with 2,3,7,8-tetrachlorodibenzo-p-dioxin (70 ng TCDD/g bw, ip) inhibits heart regeneration. Wounding the heart activates many embryonic (hand, nkx2.5, cmlc2, and sox9b) and epicardial genes (raldh2, tbx18, wt1 and tcf21). We conducted qRT-PCR experiments to determine if TCDD alters the expression of these genes during heart regeneration. Raldh2, a rate-limiting enzyme in retinoic acid synthesis and a known biomarker of the active epicardium, was significantly increased in the TCDD group compared to control, 7 days post amputation (dpa). Activation of the epicardium is necessary for proper cell migration in the regenerating ventricle. To identify effects of TCDD on the epicardium we performed in situ hybridization at 1, 4 and 7 dpa with raldh2. The results confirmed our qRT-PCR results for raldh2, but more importantly revealed that TCDD blocks the ability of raldh2 positive epicardial cells to incorporate into the regenerating ventricular tissue. In order to understand the genome-wide response to amputation with or without TCDD treatment, we performed microarray experiments. A small group of matrix metalloproteinases (mmps) was downregulated at 6 hpa in both treatment groups. Exposure to TCDD after amputation revealed an upregulation of the matrix metalloproteinases most notably mmp9 and mmp13. This group of genes is known to be responsible for the breakdown and remodeling of the extracellular matrix (ECM). The failure to activate and/or regulate appropriate genes in order to breakdown and restore the ECM may inhibit the ability of raldh2 positive epicardial cells to invade the regenerating ventricular tissue of TCDD-treated zebrafish. Supported by NIH Grant ES012716 and UW Sea Grant.

PS 198 TCDD EXPOSURE ALTERS THE GENE EXPRESSION RESPONSE TO HEART REGENERATION IN ADULT ZEBRAFISH (*DANIO RERIO*).

M. Kim, R. E. Peterson and W. Heideman. *Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin, Madison, WI.*

2,3,7,8,-Tetrachlorodibenzo-p-dioxin (TCDD) is a global environmental contaminant that causes a wide spectrum of toxicities in vertebrates. Although most adverse effects are mediated by the aryl hydrocarbon receptor (AHR), the molecular mechanisms downstream of AHR activation are poorly understood. To gain insight into these mechanisms, we have employed a zebrafish heart regeneration model. Our previous research showed that TCDD exposure 24 h prior to partial heart amputation blocks heart regeneration, whereas TCDD exposure 24 h after heart amputation does not. We used microarray experiments to identify molecular pathways altered by TCDD exposure during the early stages of the regenerative process. Male adult zebrafish (AB) were exposed to TCDD (70 ng TCDD/g bw, ip injection) or vehicle control (50 μM phosphatidylcholine) 24 h before ventricle amputation (~20% of ventricle). At 6 hours post amputation (hpa) 3 different parts of the heart were collected for microarray analysis: the atrium, the unwounded ventricle top, and the wounded ventricle bottom. Analysis showed that heart amputation and TCDD exposure altered the expression of more than 500 genes by at least two-fold in at least one of these tissues. As expected, a set of known AHR-responsive genes were highly up-regulated by TCDD exposure. Several genes induced by amputation in the controls were significantly repressed by TCDD exposure. Gene ontology (GO) terms for these genes include biological functions such as wound healing, response to stimulus, transcription, development, transport, and metabolism. These results suggest that TCDD exposure alters the expression of early response genes that may be critical to normal heart regeneration in zebrafish. This research was supported by NIH grant ES012716 and UW Sea Grant.

PS 199 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN INDUCES PRO-HYPERTENSIVE AND -ADIPOGENIC GENE EXPRESSION IN MESENTERIC ARTERIOLES PRIOR TO THE ONSET OF HYPERTENSION.

M. K. Walker, J. A. Scott and J. R. Boberg. *Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

Exposure of mice to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces a steady rise in blood pressure after 14 d, which plateaus in hypertension (+20 mmHg) by 28 d, is associated with vascular dysfunction and is mediated by cytochrome P4501A1 (CYP1A1). We hypothesized that TCDD exposure would induce pro-hypertensive changes in gene expression in resistance arteries (i.e. vessels that contribute to blood pressure regulation) prior to the rise in blood pressure. To test this we exposed C57Bl6 male mice to control pills prepared from transgenic dough or pills containing 180 ng/kg TCDD 5 d/wk for 12 d, harvested mesenteric arterioles and associated perivascular fat, isolated RNA, and analyzed changes in gene expression using Affymetrix Expression Genome Arrays (n=3/group). We analyzed for genes that were changed ≥ 2 fold in expression (up or down) at $p < 0.05$. Using these criteria the only gene associated with the aryl hydrocarbon receptor (AHR) battery that was significantly induced was CYP1A1 (30x). In contrast, CYP1A2 was not detected, while CYP1B1, AHR repressor and AHR-regulated Phase II enzymes were not induced. TCDD also significantly induced pro-hypertensive genes likely expressed in vascular smooth muscle, including prostaglandin EP3 receptor (2.8x) and phosphodiesterase 3B (2.5x). Additionally, TCDD significantly induced a large number of genes likely expressed in perivascular fat, including the pro-hypertensive gene angiotensinogen (3x), the pro-adipogenic transcription factor peroxisome proliferator activated receptor gamma (Pparg, 3.8x) and numerous Pparg downstream targets. Previous studies have shown that overexpression of angiotensinogen only in adipose can induce hypertension. Thus, our future studies will assess the tissue-specific induction of these gene targets and the degree to which TCDD-induction of angiotensinogen mediates the subsequent development of hypertension.

PS 200 GLUCOCORTICOID INDUCED LEUCINE ZIPPER PREVENTS APOPTOSIS BY CARDIAC TOXINS.

D. C. Aguilar, J. Strom, B. Xu and Q. Chen. *Pharmacology, University of Arizona, Tucson, AZ.*

Apoptosis plays a critical role in toxicity of doxorubicin (Dox) and a number of cardiac toxins. Molecules preventing apoptosis will be useful for reducing cardiac toxicity. Works from our laboratory have found that glucocorticoids are cytoprotective with isolated cardiomyocytes in culture and with the myocardium of experimental animals in vivo. Glucocorticoids induce 180 genes in cardiomyocytes, among which is Glucocorticoid Induced Leucine Zipper (GILZ). Corticosterone(CT) induces GILZ gene in a time and dose-dependent manner by transcriptional activation in cardiomyocytes. GILZ is also inducible by progesterone and retinoic acid, both of which induce cell survival response in cardiomyocytes. When GILZ gene is overexpressed in cardiomyocytes by transient or stable transfection, we observed an inhibition of apoptosis induced by Dox but not necrosis. GILZ overexpression causes elevated levels of Bcl-xL protein but not bcl-2, Bak, Bax or p21. The increase of Bcl-xL protein is independent of Bcl-xL mRNA, since GILZ expression did not cause significant elevation of Bcl-xL mRNA, suggesting that GILZ regulates Bcl-xL protein increase at translational or posttranslational level. Bcl-xL protein stability was determined using cyclohexamide treatment in cardiomyocytes transfected with full length GILZ or empty vector. GILZ transfected cells show an increase of Bcl-xL protein stability. These findings suggest that GILZ is a cytoprotective molecule and the mechanism of such cytoprotection involves Bcl-xL protein stabilization.

PS 201 IS STRUCTURAL DAMAGE TO THE HEART ASSOCIATED WITH FUNCTIONAL CHANGES?

L. Ewart, A. Hargreaves, T. Hammond, P. Lainee, J. Harleman and J. Valentin. *AstraZeneca, Macclesfield, United Kingdom.*

Human cardiotoxicity has resulted in a number of drugs being withdrawn from the market. Safety pharmacology runs highly sensitive animal models that investigate multiple cardiovascular endpoints. Combined with histopathological examination, an integrated risk assessment can be obtained on each drug destined for man. Here we have performed a meta-analysis of more than 100 proprietary small molecules to address the hypothesis that structural damage to the heart is frequently complemented by findings of functional perturbation. Heart rate (HR), mean arterial blood pressure (MABP) and left ventricular dP/dtmax measurements from conscious or anaesthetised dogs in single dose studies were aligned with histopathological findings of structural cardiotoxicity obtained from repeat dose studies (ranging

from 5 to 28 days in duration) in either rodent or non-rodents. The drugs assessed belonged to different pharmacological classes, were of varying molecular weights, had different routes of administration and were being developed for a variety of disease indications. The incidence of cardiotoxicity (e.g. myocardial necrosis, myocarditis, hypertrophy and myocardial degeneration) associated with a functional perturbation was low (10%). Cardiotoxicity resulted when MABP decreased and HR increased (63%) or with decreased HR alone (37%). There was no structural or functional change in 46% of the drugs studied but interestingly, the remaining 44% had changes in at least 1 of the measured parameters but no histopathology. There were no drugs that showed structural damage only. Taken together, these results indicate that there is an association between the independent findings of functional and structural change within the heart. This analysis also indicates that drugs with no effect on HR, MABP or dP/dtmax in acute studies are likely to also show no structural damage upon repeat dosing. Increasing our understanding of drugs that cause functional change in the absence of structural change is critical in our quest to reduce drug attrition due to cardiotoxicity.

PS 202 USE OF NATRIURETIC PEPTIDES TO DETECT COMPOUND RELATED CHANGES IN BLOOD PRESSURE IN RODENTS.

S. K. Engle, L. Huber, D. Sall, M. Uhlik and D. E. Watson. *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN.*

Cardiovascular toxicity is a serious adverse event observed in pre-clinical studies during drug development. Myocardial degeneration is efficiently detected by markers of membrane leakage, such as cardiac troponin I. In contrast, functional changes in the cardiovascular (CV) system, such as blood pressure, are not easily detected in rodent studies conducted in early drug development. Altered hemodynamic parameters can, over time, lead to undesirable adaptations, such as cardiac hypertrophy, or myocardial degeneration. Natriuretic peptides (NP), regulate blood pressure through excretion of salt and water, and vasorelaxation, and are potential biomarkers of change in blood pressure. We demonstrated the use of NPs in serum or plasma as biomarkers of blood pressure changes in mice and rats. The direction of change in blood pressure and NTproANP concentration were inversely related. Treatment of mice with linifanib (25 mg/kg), a compound known to cause increased mean arterial blood pressure (20-30 mmHG) in mice, caused a three-fold decrease in NTproANP concentration ($p<0.05$). Treatment of mice with a PPAR α/γ dual-agonist (50 mg/kg) known to cause decreased blood pressure in rats, caused a 7-fold increase in NTproANP concentration ($p<0.05$). Administration of nifedipine (30 mg/kg) to rats caused decreased blood pressure (15-20 mmHg) and increased NTproANP concentration 2-3 fold ($p<0.05$). Administration of a compound which inhibits a metabolic enzyme caused increased blood pressure (20-30 mmHg) and a 2-3 fold decrease in NTproANP ($p<0.05$) in rats. Treatment of rats with L-NAME (30 mg/kg) caused increased blood pressure (15-20 mmHg) and a 2-fold increase in NTproBNP ($p<0.05$). Measurement of NP concentrations in serum or plasma is a rapid, cost-effective, and minimally invasive method for detecting important changes in CV function, and has been useful for prioritizing molecules for advancement into more expensive toxicology and safety pharmacology studies during drug development.

PS 203 THE C-TERMINUS OF THE CARDIAC ELECTRONEUTRAL SODIUM BICARBONATE COTRANSPORTER HHNBCN1 IS CYTOTOXIC.

N. Magilnick, N. Abuladze, P. Ge, I. Atanasov and A. Pushkin. *D. Geffen School of Medicine at the University of California Los Angeles, Los Angeles, CA.* Sponsor: W. Dekant.

The electroneutral sodium bicarbonate cotransport is shown to play an important role in the intracellular pH (pHi) and sodium (Nai) regulation in the heart and other organs. Despite the functional importance of the human heart sodium bicarbonate cotransporter (hhNBCn1), its high-resolution structure is unknown and that significantly limits our understanding of the molecular mechanisms that regulate pHi and Nai in cardiac cells. Contrary to the electrogenic sodium bicarbonate cotransporter NBCe1, the expression of hhNBCn1 was toxic for both the mammalian (HEK293) and yeast (Sf9) cells. hhNBCn1 was predominantly expressed as a homodimer similar to NBCe1 as shown by size-exclusion chromatography and electron microscopy. The expression level of an inactive H1083A mutant of hhNBCn1 was higher than the wild type (wt) hhNBCn1 but did not reach the expression level of NBCe1 suggesting that factors other than the functional activity may be responsible for wt-hhNBCn1 toxicity. The mutation did not affect the protein oligomerization state. A shortened version of hhNBCn1 missing its cytoplasmic C-terminus (amino acids 1121-1214) that had an oligomeric state similar to wt-hhNBCn1 was expressed at a significantly higher level than wt-hhNBC1 indicating that the C-terminus is involved in mediating wt-hhNBCn1 toxicity. In agreement with this assumption the His6-tagged C-terminus was toxic to HEK293, Sf9 and E. coli cells. The C-terminal hhNBCn1-specific antibody immunoprecipitated an ~20 kDa protein from the HEK293 cells expressing wt-hhNBCn1. Our data suggest that the C-terminus of hhNBCn1 is not important for the protein oligomerization and may be involved in mediating the toxicity of hhNBCn1 expressed in mammalian cells.

PS 204 USING SOD2 HETEROZYGOUS MOUSE MODEL TO STUDY THE MECHANISM OF DOXORUBICIN INDUCED CARDIAC TOXICITY.

W. Hu, W. Collette, L. Liu, M. Hemkens, J. Heyen, S. Aquirre, L. Marroquin, B. Jessen and V. Torti. *DSRD, Pfizer, San Diego, CA.*

Superoxide dismutase (SOD) is a family of antioxidant enzymes that scavenge superoxide free radicals and modify oxidant balance in the cell. SOD2 encodes a mitochondrial matrix protein shown to protect the heart from oxidative injury and ischemic damage. Mice lacking SOD2 die shortly after birth, however, SOD2+/- mice have a normal lifespan and minimal phenotypic defects. In this study, we utilized the SOD2+/- mouse model to test the functional significance of SOD2 after doxorubicin treatment, and evaluated the utility of SOD2+/- mouse model for studying the mechanism of cardiotoxicity. SOD2+/- mice and strain-matched wild-type mice were treated with doxorubicin at 5 or 10mg/kg/week for 8 weeks. Endpoints measured in this study included: heart/body weight, clinical chemistry, echocardiography, and histopathology. A portion of the heart was taken for whole genome transcriptomics analysis and a portion of the liver was homogenized for glutathione content assay. Multifocal myocardium fibrosis was observed in SOD2+/- mice treated with 10mg/kg doxorubicin, but not in wild-type mice. Doxorubicin treatments in SOD2+/- mice for 8 weeks resulted in oxidative stress as shown by dose dependent glutathione depletion. The reduced level of SOD2 leaves the heart more susceptible to oxidative injury and results in gene expression changes indicative of pro-inflammatory response, cell death, cardiac remodeling and potential hemolysis in response to doxorubicin, events less significant in wild type mice. Even at the low dose where morphological damage was not evident, doxorubicin induced acute phase proteins and stress response in SOD2+/- mice, but not in wild-type. These findings demonstrate that the SOD2+/- mouse model may be useful to study cardiac toxicity mediated via oxidative stress and mitochondrial dysfunction; it may provide a more sensitive (left shift of dose response) and predictive (early onset) measurement of cardiac toxicity than traditional animal models.

PS 205 INDUCTION OF CARDIOTOXICITY BY PERFLUOROOCTANOIC ACID IN AN AVIAN MODEL.

Q. Jiang and J. DeWitt. *East Carolina University, Greenville, NC.*

Perfluorooctanoic acid (PFOA) is a widespread and persistent environmental contaminant and a multisystem toxicant. In the US, human exposure is mainly via drinking water and average serum levels are 4ng/ml. As lab studies have indicated that developmental toxicity is an endpoint of concern, we assessed PFOA's effects on heart development in a chicken embryo model. Fertile eggs were air cell injected prior to incubation with 0.5, 1, or 2 mg PFOA/kg egg weight in safflower oil vehicle. Embryos were euthanized at day 19 of incubation; hearts were rapidly removed, relaxed in ice-cold saline, and fixed in 10% neutral buffered formalin. Ventricles were embedded in paraffin after being separated from atria. Hearts were sectioned at 6μ with a rotary microtome and morphology was evaluated with standard histology and immunohistochemistry on myocardium dense layer. Preliminary data suggest that developmental exposure to PFOA decreases right ventricular wall (RVW) whole thickness as well as myocardium layer thickness. Staining with Hematoxylin and eosin revealed that RVW was 20.2% thinner in the 1 mg/kg group relative to vehicle control ($P<0.05$). RVW thickness in the 2 mg/kg group was 18.06% thinner relative vehicle control, but it was not statistically significant ($P = 0.06$). Elevated mortality was observed in the 2 mg/kg group, which may have selectively removed sensitive embryos and prevented detection of the difference. Staining myocardium dense layer with anti-myosin antibody mf-20 produced similar results in the 1mg/kg group. Decreased RVW thickness indicated heart morphology is altered by PFOA. Decreased myocardium dense layer, the major contributor of contractility, showed that function could be affected, as the right ventricle contributes 60% of total cardiac output in the embryonic stage. This study indicates that developmental exposure to PFOA in an avian model affects heart morphology.

Additional work with hatchling chickens will evaluate cardiac function with Doppler ultrasound and additional work with chicken embryos is planned to evaluate the mechanism of PFOA-induced cardiotoxicity.

PS 206 CHRONIC OVEREXPRESSION OF RECEPTOR TYROSINE KINASE ERBB2 INDUCES CELLULAR OXIDATIVE STRESS IN THE HEARTS OF MICE.

F. Belmonte[1], Y. Xu[1], S. Polina[1], C. Steenbergen[2], S. Das[2], X. Guo[1], S. Pin[1], D. Bedja[1] and K. Gabrielson[1]. *[1]Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD and [2]Cardiovascular Pathology, Johns Hopkins University, Baltimore, MD.*

Anti-erbB2 (Herceptin) and doxorubicin are cancer therapy drugs that when given together can cause synergistic cardiac toxicity. Herceptin targets erbB2- (a receptor tyrosine kinase, Her2/neu) found overexpressed in breast cancer. Radioactive Herceptin binds to the hearts in some women during cancer therapy with poor cardiac outcomes. The regulation of erbB2 in the heart is not well understood. We previously found that doxorubicin induces erbB2 in rat hearts possibly predisposing the heart to off-target toxicity to anti-erbB2. We hypothesize that doxorubicin upregulates erbB2 through an oxidative stress-mediated pathway. To support this, neuregulin (ligand for the erbB family) was previously found to upregulate mRNA transcripts for multiple oxidative stress defense proteins in cell culture cardiomyocytes. To further understand the role of cardiac erbB2 in vivo, we developed a transgenic mouse model that has chronically elevated levels of cardiac erbB2 under aMHC promoter. In preliminary studies, erbB2 overexpression in mouse hearts results in upregulation of cytosolic thioredoxin-1, mitochondrial thioredoxin-2 and glutathione reductase proteins. Induction of these particular proteins suggests that chronic erbB2 overexpression is either reducing, or conversely, producing oxidative stress. Using an OxyBlot method to detect oxidative stress, whole cell lysates from hearts of the transgenic mice showed increased oxidative stress compared to wild type mice. ErbB2 overexpression also induces an upregulation of uncoupling protein 3, indicating that the source of oxidative stress may be the mitochondria. Thus, we hypothesize that chronic erbB2 signaling induces oxidative stress in cardiomyocytes. Using these findings, we hope to understand what patient population is at risk for synergistic toxicity and the role of erbB2 in oxidative stress protection or induction in cardiomyocytes.

PS 207 RECOVERY OF CYTOCHROME C OXIDASE ACTIVITY IS REQUIRED FOR DIETARY COPPER SUPPLEMENTATION-INDUCED REGRESSION OF CARDIAC HYPERTROPHY IN MICE.

L. Zhan[1], L. Hussain[1], Y. Yao[1], W. Feng[2], K. Bourcy[1], J. Eaton[2], W. Johnson[3], C. Moraes[4] and Y. Kang[1]. *[1]Pharmacology & Toxicology, University of Louisville, Louisville, KY, [2]Medicine, University of Louisville, Louisville, KY, [3]Human Nutrition Research Center, U.S. Department of Agriculture, Grand Forks, ND and [4]Cell Biology and Anatomy, University of Miami Miller School of Medicine, Miami, FL.*

Sustained pressure overload causes cardiac hypertrophy and transition to heart failure, along with depressed cytochrome c oxidase (CCO) activity. Dietary supplementation with physiologically relevant levels of Cu restores CCO activity and reverses cardiac hypertrophy induced by pressure overload. To test the hypothesis that CCO activity is essential for Cu supplementation-induced regression of hypertrophic cardiomyopathy, a cardiac-specific COX10 conditional knockout (KO) mouse model was used,which was generated by crossing a LoxP-tagged COX10 mouse with a transgenic mouse expressing a tamoxifen-inducible Cre recombinase under the control of cardiac a-myosin heavy chain promoter. Mice were fed an AIN-93 diet containing adequate Cu (6 ppm) and subjected to transverse aortic constriction (TAC) or sham surgery. One week after the surgery, the mice were administered with 20 mg/kg tamoxifen daily for 5 consecutive days. COX10 KO mice showed a 60-80% reduction in cardiac CCO activity after the tamoxifen treatment, but wild-type (WT) mice did not. Five weeks after the surgery, some mice were switched to a Cu supplementation diet (20 ppm Cu) and others remained on their previous diet for 4 more weeks. Mice were harvested at wk 5 or wk 9 after the surgery. There was no significant difference in TAC-induced cardiac hypertrophy between WT and COX10 KO mice. Dietary Cu supplementation caused a regression of cardiac hypertrophy and fibrosis, and functional recovery in the WT mice, but did not affect the pathological progression in the COX10 KO mice. These results indicate that CCO is essential in Cu-induced regression of hypertrophic cardiomyopathy. Supported in part by a NIH grant (HL-63760 to YJK).

PS 208 THE INVOLVEMENT OF VIMENTIN IN THE CU INDUCED REGRESSION OF HYPERTROPHY IN HUMAN CARDIAC MYOCYTES.

K. S. Bourcy[1], W. Feng[2] and Y. Kang[1]. *[1]Pharmacology & Toxicology, University of Louisville, Louisville, KY and [2]Medicine, University of Louisville, Louisville, KY.*

Previous studies have shown that copper (Cu) supplementation at physiologically relevant levels reverses cardiac myocyte hypertrophy induced by phenylephrine (PE), and that this effect was VEGF-dependent. Yet, the amount of VEGF in the media was unchanged, but we observed that Cu caused an increase in the ratio of VEGFR1:VEGFR2 as well as an increase in PKG-1 activity, which is associated with the regression of cardiac myocyte hypertrophy. The present study was undertaken to test the hypothesis that VEGFR1 is associated with PKG-1 and their association is involved in Cu mediated regression of cardiomyocyte hypertrophy. Human cardiac myocytes (HCM) in cultures were exposed to phenylephrine (PE) at a final concentration of 100 μM for 48 hours to induce cell hypertrophy. Copper sulfate at a final concentration of 5 μM was added to the hypertrophic HCM cultures for 24 hours with the concomitant presence of PE to reverse the hypertrophy. Both hypertrophic and hypertrophic-reversed HCM cells underwent immunoprecipitation using anti-VEGFR1 antibody or anti-PKG-1 antibody. The immune complex underwent gel-electrophoresis separation and Western blotting and LC-MS/MS analysis. Proteomic analysis identified vimentin, a known PKG-1 binding protein, in the immune complex associated with VEGFR1. Gene silencing of vimentin blocked Cu-induced regression of cardiac myocyte hypertrophy and decreased PKG-1 activity. This study thus demonstrates the likely association between both VEGFR1 and PKG-1 with vimentin, and that vimentin plays a critical role in copper regression of cardiac myocyte hypertrophy.

PS 209 DIRECT EFFECTS OF NRTIS ON MITOCHONDRIA CONTRIBUTE TO ENDOTHELIAL DYSFUNCTION.

M. C. Glover, S. Xue, V. Y. Hebert, J. H. Zavecz and T. R. Hebert. *Pharmacology, Toxicology, and Neuroscience, Lousiana State University Health Sciences Center, Shreveport, LA.*

Nucleoside reverse transcriptase inhibitors (NRTIs) are integral components of combination therapies for the treatment of HIV infection. Chronic NRTI treatment, however, has been associated with cardiovascular complications, including atherosclerosis. Our prior studies demonstrated that a number of NRTIs induce endothelial dysfunction, an initiating factor in the development of atherosclerosis, and that this dysfunction is due, at least in part, to mitochondrial toxicity. One widely held theory is that NRTIs inhibit mitochondrial DNA polymerase-γ, leading to depletion of mitochondrial DNA. However, our in vitro studies, demonstrated alterations in ATP production, ROS generation, and activity of the mitochondrial electron chain complexes within hours of NRTI treatment, suggesting a direct effect on mitochondrial function. The purpose of this study was to investigate the mechanisms of short-term vs chronic NRTI-induced mitochondrial toxicity. Wildtype (WT) compared to transgenic (Tg) mice overexpressing MnSOD were treated for 1 wk or 6-8 wks with AZT (zidovudine), TDF (tenofovir disoproxil fumarate), or 3TC (lamivudine). Vessel reactivity experiments were performed to assess endothelial function following treatment. Phosphorylation studies on liver mitochondrial complex I isolates were performed to determine alterations in the regulation of complex I activity, and by extension, mitochondrial electron transport. WT but not MnSOD Tg mice treated for 6-8 wks demonstrated endothelial dysfunction. Moreover, although short-term treatment with NRTIs did not impair endothelial function, it did alter phosphorylation of at least one complex I subunit. These results support the hypothesis of a direct mechanism of mitochondrial toxicity in addition to indirect effects on mitochondrial DNA replication. Phosphorylation studies on complex I isolates from Tg mice are ongoing, as well as electron microscopy of treated vs. untreated endothelial mitochondria.

PS 210 ANG II INDUCED PKC/NOX ACTIVATION-ASSOCIATED NITROSATIVE DAMAGE PLAYS A CRITICAL ROLE IN CHRONIC ALCOHOL-CAUSED CARDIAC CELL DEATH AND CARDIOMYOPATHY.

Y. Tan, Z. Zhou and L. Cai. *Pediatrics/Medicine, University of Louisville, Louisville, KY.*

Alcohol induced cardiac cell death is causative to cardiomyopathy, but the mechanisms responsible for alcoholic cardiac cell death remain largely unknown. We hypothesize that alcohol-induced nitrosative stress plays a critical role in cardiac cell death and cardiomyopathy. To test this hypothesis, male C57BL/6 mice were pair-fed an alcohol or isocaloric maltose dextrin liquid diet for 2 months. Alcohol feeding caused significant cardiac cell death and nitrosative damage (shown by TUNEL

positive cells, caspase-3 activation and protein nitration) and cardiac remodeling (shown by hypertrophy and fibrosis). Mechanistic study in which cardiac H9c2 cells were treated with 100-400 mM alcohol for 24h revealed that alcohol exposure induced cell death, nitrosative damage and fibrosis, along with NADPH oxidase p47phox (NOX) activation in a dose-dependent manner. Pre-treatment of alcohol-treated cells with NOX inhibitor (apycinin), peroxynitrite scavenger (uriate), NOS inhibitor (L-NAME) and superoxide dismutase mimic (MnTMPyP) significantly abolished alcohol induced cell death. Furthermore, exposure to alcohol significantly up-regulated the expression of angiotensin II (AngII) and its type 1 receptor (AT1) in vitro and in vivo, either PKCα/β1 inhibitor or AT1 blocker completely prevented alcoholic NOX activation, and AT1 blocker inhibited the expression of PKCβ1, implying that alcoholic NOX activation is dependent of PKCα/β1 activation via AT1.To validate the in vitro findings, alcohol-fed and pair-fed mice were treated with MnTMPyP (5 mg/Kg) daily for 2 months, which did not change blood pressure, alcohol-induced cardiac PKC and NOX activation, but significantly prevented alcohol-induced cardiac nitrosative damage and cell death, along with a prevention of cardiac fibrosis and dysfunction. These results suggest that alcohol-induced cardiac cell death is mediated by up-regulated Ang II interaction with AT1 to induce PKCα/β1-dependent NOX activation, and the cardiac cell death plays a critical role in the development of alcoholic cardiomyopathy.

PS 211 ASSESSMENT OF THE VARIATION IN TROPONIN I LEVELS IN THE DOG DURING THE PERIOD PRIOR TO TOXICOLOGICAL STUDY DOSING.

C. J. Betts, T. Child, S. J. Bickerton, M. Moores, N. Derbyshire, H. T. Thomas, M. C. Jacobsen and S. Robinson. *Safety Assessment, AstraZeneca, Macclesfield, Cheshire, United Kingdom.*

Cardiotoxicity is major cause of attrition of novel compounds during preclinical and clinical development. Thus cardiovascular toxicity is a primary safety concern for the pharma industry and there is a drive to progress the assessment and use of predictive biomarkers to reduce attrition in late stage development. Cardiac troponin is a direct marker for cardiac myocyte damage and is used in the clinic for monitoring cardiac injury. This marker can be readily assessed in preclinical toxicity study species using species-specific ELISA methodologies or automated analysers developed for the assessment of troponin in the clinic. The objective of this study was to assess the levels of troponin I (TNI) in the period prior to the start of compound administration. Animals were acclimatised for at least 3 weeks prior to dosing and were sham dosed over the period of sample collection. Twelve female dogs were sampled daily for 7 days prior to the start of dosing by oral gavage. Blood was taken at the same time each day and analysed using an Ultrasensitive human TNI assay validated for use with dog plasma on the Siemens Centaur CP analyser. In the majority of animals TNI levels remained close to the assay limits of detection (0.006 ng/mL) for the period prior to study start. However, 5 out of 12 animals demonstrated sporadic, marked increases in TNI to levels considered indicative of cardiac toxicity, but which returned to baseline values at the next sample point. This analysis highlights that plasma levels of TNI can vary markedly in individual animals during the acclimatisation period prior to toxicological study start. The cause of this release is not clear, although it coincides with the onset of handling and dosing procedures. Thus to prevent sporadic, non-dose related increases in TNI during the early dosing period animals should be acclimatised over an extended period of time with sham dosing prior to study start if possible.

PS 212 EGCG DECREASES POLYCHLORINATED BIPHENYL-INDUCED EXPRESSION OF CYP1A1 AND ENDOTHELIAL INFLAMMATORY PARAMETERS *VIA* INDUCTION OF ANTIOXIDANT ELEMENTS.

B. Hennig[1], S. Han[1] and M. Toborek[2]. [1]*Animal and Food Sciences, University of Kentucky, Lexington, KY and* [2]*Department of Neurosurgery, University of Kentucky, Lexington, KY.*

Epigallocatechin gallate (EGCG) protects against vascular diseases such as atherosclerosis via its antioxidant and anti-inflammatory functions. Polychlorinated biphenyls (PCB) are persistent and widespread environmental contaminants that induce oxidative stress and inflammation in vascular endothelial cells. Although PCBs are no longer produced, they are still detected in human tissues and thus a risk for vascular dysfunction. We hypothesized that EGCG may protect endothelial cells against PCB-induced cell damage via its antioxidant and anti-inflammatory properties. To test this hypothesis, primary vascular endothelial cells were pretreated with EGCG (25 and 50 μM), followed by exposure to coplanar PCB126 (0.25 μM). Exposure to PCB126 significantly increased cytochrome P450 1A1 (Cyp1A1) mRNA and protein expression and superoxide production, events which were significantly attenuated following pretreatment with EGCG. Similarly, EGCG also attenuated PCB126-mediated induction of adhesion molecules such as monocyte chemotactic protein-1 (MCP-1). Furthermore, EGCG decreased endogenous or base-line levels of Cyp1A1 and MCP-1 in endothelial cells. Most of all, treatment of EGCG upregulated expression of phase II antioxidant enzymes, glutathione S transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), in a dose-dependent manner via Nrf2 signaling. Pretreatment of all-trans retinoic acid, an inhibitor for antioxidant response element (ARE)-driven gene expression, resulted in an increase of Cyp1A1 and MCP-1 expression, suggesting ARE-driven antioxidant enzymes play an important role against PCB-induced inflammatory responses in endothelial cells. These data show that EGCG may reduce cardiovascular disease risks caused by PCB exposure via elevated expression of antioxidant genes. (This work was supported in part by NIH/NIEHS grant P42ES007380 and the UK AES)

PS 213 QUANTITATIVE PROTEOMICS REVEALS RAPID CHANGES IN NA+/K+ ATPASE AND NCX1 LEVELS IN ADULT ZEBRAFISH HEART FOLLOWING TCDD EXPOSURE.

K. Lanham[1], J. Zhang[2], R. Peterson[2], W. Heideman[2] and L. Li[2]. [1]*Biomolecular Chemistry, University of Wisconsin Madison, Madison, WI and* [2]*School of Pharmacy, University of Wisconsin Madison, Madison, WI.*

The hearts of zebrafish embryos and larva are acutely sensitive to TCDD. Within hours of exposure to TCDD cardiac output is reduced, which is followed by severe heart malformations and pericardial edema. Interestingly, juvenile and adult zebrafish hearts are refractory to the acute effects of TCDD exposure, but it is unknown how this occurs. One possibility is that the mechanism responsible for toxicity in the embryo is still active, but the adult has found a way to compensate for the disruption. To investigate this possibility we used a proteomic approach to determine whether there are any early alterations in the adult heart consistent with a toxic response to TCDD. Adult zebrafish were exposed to TCDD (1 ng/mL), or vehicle, for 1 hour by static water born exposure, and whole hearts were collected at 24 hours post-exposure. Quantitative mass spectrometry based on the spectral counting approach was employed to evaluate the protein expression changes induced by TCDD. Using the NSAF (normalized spectral abundance factor) approach we found 105 proteins with altered abundance following TCDD exposure, 60 of these were over expressed (>1.5 fold) and 45 were under expressed (<0.5 fold). Notable among our findings was the down-regulation of multiple Na+/K+ ATPase alpha subunit isoforms together with the simultaneous up-regulation of the Na+/Ca++ exchanger (NCX1) by TCDD. This combination of decreased Na+/K+ ATPase availability with an increase in NCX1 could result in an elevated calcium load in the heart and suggests a mechanism for the altered cardiac output observed in zebrafish embryos and larva following TCDD exposure. This work highlights the utility of mass spectrometry and proteomics in toxicity assessment, which can provide unique insight into mechanisms that may not be revealed by standard genomic approaches.
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PS 214 HSP90-BETA OVEREXPRESSION IN THE MOUSE HEART INDUCES HEART FAILURE.

Y. Xu[1], D. Bedja[1], X. Guo[1], M. Mei[1], P. Sysa Shah[1], S. Das[2], C. Steenbergen[2] and K. Gabrielson[1]. [1]*Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD and* [2]*Cardiac Pathology, Johns Hopkins University, Baltimore, MD.*

Heat shock protein 90(HSP90) is a chaperone for hundreds of proteins, aiding protein folding and protein function. HSP90 is up-regulated in the heart during doxorubicin and cyclosporin A exposure and is hypothesized to be protective. To test this hypothesis, αMHC promoter driven HSP90β transgenic mouse lines (high, moderate and low expression) were generated. Cardiac specific HSP90 over-expressing mice (OE) and wild type (WT) littermates were followed by echocardiography, histopathology, mRNA microarray, mitochondrial oxygen consumption and immunoblotting. Surprisingly, we found an inverse relationship between contractility and HSP90 expression, with the highest expression group having the lowest cardiac contractility, (fractional shortening 41.2 % OE mice versus 68% in WT mice). The decline in contractility was paralleled by myocardial necrosis and fibrosis. mRNA microarray analysis indicated that mitochondrial transcripts were markedly reduced and that function may be impaired by HSP90 OE. This was confirmed by mitochondrial isolation and oxygen consumption methods using complex I substrates since HSP90 OE mice have mitochondrial respiratory control ratios of 4 versus 8 in WT. Since HSP90 is toxic, we hypothesized that the cell would negate its effects by acetylation, a known modification of HSP90 to reduce its activity in cancer cells. Additionally, a disabled HSP90 chaperone would induce a faster protein turnover due to accelerated protein proteosomal degradation. Western blotting methods confirmed significant global ubiquitination and acetylation in the hearts of HSP90β TG versus WT mice. Our in vivo results reveal that prolonged HSP90β OE disrupts the homeostasis of chaperone and client proteins with a dysregulation of cellular ubiquitination and acetylation.

PS 215 HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES FOR ASSESSING DRUG INDUCED CARDIAC ARRHYTHMIAS.

B. Anson[1], J. Ma[1], L. Guo[2], S. Fiene[1], R. Adams[2], T. Weiser[3], T. Singer[3] and K. Kolaja[2]. *[1]Cellular Dynamics International, Madison, WI, [2]Non-Clinical Safety, F. Hoffman-La Roche, Nutley, NJ and [3]Pharmacology Research and Early Development, F. Hoffman-La Roche AG, Basel, Switzerland.*

The potential for drug-induced cardiac arrhythmias is a vital component of the toxicological and safety pharmacological profile of new chemical entities (NCEs). Cardiomyocytes contain multiple ion channels and ion transporters and cardiac electrical activity arises from the temporal and spatial activity of these proteins. Commonly employed methods fail to recapitulate the human condition as the predominant systems typically express a single channel without ancillary proteins and may therefore miss exacerbating or compensatory protein interactions, while animal models may ultimately miss species-specific effects. Human induced pluripotent stem cell (hIPSC) -derived cardiomyocytes provide a relevant human system in which to assess the arrhythmogenic nature of an NCE. We present data characterizing the electrophysiological profile of hIPSC-derived cardiomyocytes and demonstrating their suitability for assessing NCE-induced arrhythmogenicity. Single cell voltage and current clamp experiments demonstrated the presence of multiple endogenous ionic currents present in human cardiomyocytes including I_{Na}, I_{Ca}, I_{to}, I_{Kr}, I_{f}, and I_{K1} while drug application produced the expected changes in action potential phenotypes including an approximate 70% increase in duration in response to hERG channel block (100nM of E-4031), 80% decrease in duration in response to Ca^{2+} channel block (100nM nifedipine), and a 60% slowing of the action potential upstroke in response to Na^{+} channel block (10μM TTX). These results were verified through organotypic extracellular recordings made with microelectrode array (MEA) techniques that also demonstrated a 35% increase in field potential duration in response to block of IKs by 10μM chromanol 293B. This dataset demonstrates that hIPSC-derived cardiomyocytes are a suitable and novel human-based model for assessing NCE arrhythmogenic potential.

PS 216 ISIS 416858, AN ANTISENSE INHIBITOR OF FACTOR XI, IS WELL TOLERATED AND PRODUCES NO RISK OF BLEEDING IN THE CYNOMOLGUS MONKEY FOR UP TO 13 WEEKS OF TREATMENT.

H. S. Younis[1], J. Crosby[2] and S. P. Henry[1]. *[1]Preclinical Development, ISIS Pharmaceuticals, Carlsbad, CA and [2]Drug Discovery, ISIS Pharmaceuticals, Carlsbad, CA.*

Complications associated with increased bleeding risk are the main limitations with current anticoagulant therapy. A strategy to produce sufficient anticoagulant properties with reduced bleeding risk may be the inhibition of Factor XI in the intrinsic coagulation cascade. The objective of this work was to determine the safety profile of ISIS 416858, a 2'-methoxyethoxy antisense oligonucleotide inhibitor of Factor XI, with special focus on assessing bleeding risk. Cynomolgus monkeys were administered ISIS 416858 (4, 8, 12 and 40 mg/kg/wk, SC) for up to 13 weeks. ISIS 416858 produced a dose-dependent reduction in systemic Factor XI concentrations and a concomitant increase in aPTT. Plasma Factor XI was reduced by 80% at 4 weeks of treatment (40mg/kg/wk) that resulted in a 33% increase in aPTT by 13 weeks. No effects on PT or platelets were observed during this time period confirming that the effects of ISIS 416858 on Factor XI inhibition were limited to the intrinsic coagulation cascade. Skin laceration bleeding time, a measure of bleeding risk, was unchanged following 13 weeks of treatment. ISIS 416858 produced typical changes anticipated for an antisense oligonucleotide in the monkey including acute and transient complement activation, basophilic granules and mononuclear cell infiltrates in multiple tissues, which increased in incidence and severity in a dose-dependent manner. Collectively, these results suggest that ISIS 416858 produces antisense inhibition of Factor XI, is well tolerated and produces no risk of bleeding at clinically relevant doses.

PS 217 BENZO[A]PYRENE AUGMENTS INFLAMMATORY AND PROTEOLYTIC CHANGES IN MOUSE MODEL OF ABDOMINAL AORTIC ANEURYSM.

P. A. Prins[1], P. R. Perati[1], A. Ramesh[2], Z. Guo[2] and U. K. Sampson[1]. *[1]Cardiovascular Medicine, Vanderbilt University, Nashville, TN and [2]Meharry Medical College, Nashville, TN.*

Background: Benzo[a]pyrene [B(a)P] is an abundant environmental polycyclic aromatic hydrocarbon (PAH) known to promote atherosclerosis—a predisposing factor for the development of abdominal aortic aneurysms (AAA). Since experimental evidence suggests that inflammation and enzymatic degradation of the vascular wall are central to the pathobiology of AAA, we sought to determine the effects of BaP exposure on these processes. Methods: A total of 112 male, 5 weeks of age, Apo E-/- mice were evaluated in 4 experimental groups: B(a)P (n=39), AngII (n= 30), B(a)P+AngII (n=29) and control (n=24). Exposure to B(a)P (0.71mg/kg/day x 60days) was via oral gavage and AngII exposure (1000ng/kg/min x 2 wks) occurred via subcutaneous osmotic mini pumps. In the B(a)P+AngII group, AngII exposure overlapped with the last two weeks of B(a)P treatment. Results: There were no incidence of AAA in either control or B(a)P groups. There was no difference in the number of intact (Ni) and ruptured (Nr) AAA between the AngII (Ni = 10, Nr = 7) and B(a)P + AngII (Ni = 8, Nr = 8) groups. Intact AAA from mice in the B(a)P+AngII group were significantly larger in cross-sectional diameter (2.30 ± 0.09, N=8) compared to those of the AngII group (1.9 ± 0.09, N=10) ($p < 0.05$). Immunohistochemical and molecular evaluation of aortic tissue from B(a)P+AngII, and AngII treatment groups demonstrated higher levels of tumor necrosis factor alpha (TNF-α) and cyclophilin A (Cyp A) proteins, and of matrix metalloproteinase (MMP9) in the B(a)P+AngII group. Accordingly, serum inflammatory cytokine levels (TNF-α) and interleukin (IL-6) were higher in the B(a)P+AngII group. Conclusions: These findings suggest that B(a)P potentiates the inflammatory and proteolytic processes that attend AAA formation. Given the abundance of B(a)P (e.g. in charcoaled foods and smoke), the possible role of B(a)P in AAA evolution merits further evaluation for mechanistic insights.

PS 218 ARYL HYDROCARBON RECEPTOR AGONIST - PCB 77 - INCREASES THE INCIDENCE AND SEVERITY OF ABDOMINAL AORTIC ANEURYSM.

V. Arsenescu, R. Arsenescu and L. Cassis. *GCNS, University of Kentucky, Lexington, KY.* Sponsor: H. Swanson.

Rationale: Male sex and smoking are the main risk factors for abdominal aortic aneurysm (AAA). PCB 77, a dioxin-like component of cigarette smoke, triggers AhR dependent inflammatory response in the cardiovascular system. We hypothesized that PCB77 will induce inflammation in the perianeurysmal adipose tissue and promote the development of Angiotensin II (AngII) - induced AAA. Methods: We injected male, apoE-/- mice with vehicle or PCB 77 (150mmol/Kg, i.p) at the beginning and at the end of 1 month AngII infusion. We monitored body weight, blood pressure and the aneurysm formation and progression using ultrasonography. The incidence of abdominal aortic aneurysm was analyzed in a cohort of patients with/without exposure to cigarette smoke. CYP1A1 induction was determined in aortic wall specimens from patients that underwent AAA surgical repair. Results: PCB 77 increased body weight by expanding visceral adipose tissue and liver mass in AngII + PCB 77 - infused mice. In addition, total cholesterol and free fatty acids (NEFA) were increased in mice receiving PCB 77. AAA incidence and severity were increased in mice exposed to PCB 77 (9 out of 10) compared to AngII group (5 out of 11). PCB77 was preferentially accumulated in the mesenteric fat. Furthermore, PCB 77 treatment induced proinflammatory adipokines, Angiotensin, AT1 and macrophage accumulation in the perianeurysmal adipose tissue while decreasing the antiinflammatory adiponectin. Patients exposed to cigarette smoke had twice the incidence of AAA and it was correlated with high CYP1A1 expression in the aneurysmal aortic wall. Conclusions: Persistent exposure to a dioxin like toxicant was associated with increase AAA severity and mortality. Metabolic syndrome and inflammatory response in the perivascular fat could explain this association. The strong correlation between smoking and AAA in human subjects further supports our observations. Smoking cessation and avoidance of fatty foods containing dioxin-like toxicants represent viable approaches for prevention and early, non-surgical management of patients with AAA.

PS 219 NNK, A TOBACCO-SPECIFIC CARCINOGEN, INHIBITS THE EXPRESSION OF LYSYL OXIDASE: A TUMOR SUPPRESSOR GENE.

Y. Zhao, S. Gao, J. Zhou, P. Toselli and W. Li. *Biochemistry, Boston University School of Medicine, Boston, MA.*

4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific carcinogen, is believed to contribute to the cancer burden in cigarette smokers. To evaluate NNK effects on the expression of lysyl oxidase (LO), a tumor suppressor gene, we examined this enzyme at promoter, mRNA, protein and catalytic levels in NNK-treated rat fetal lung fibroblasts (RFL6). Exposure of cells to NNK at 10, 30, 100, and 300 μM for 48 h reduced LO transcript levels to 79.3, 56.4, 8.6 and 1.8% of the control, respectively. NNK decreased levels of all LO protein species including the 46 kDa preproenzyme, the 50 kDa proenzyme and the 32 kDa mature enzyme, in a dose-dependent manner. Notably, the 50 kDa and the 32 kDa species of LO were more sensitive to NNK. Although 300 μM NNK, markedly de-

creased level in the 46 kDa preproenzyme, under same conditions, there was no detectable amount of the 50 kDa proenzyme and the 32 kDa mature enzyme suggesting NNK perturbation of the LO processing to its mature form. Moreover, NNK also induced a dose-dependent inhibition of LO activities in conditioned media of treated cells, consistent with its inhibitory effects on the mRNA and protein levels. At the promoter level, NNK suppressed LO promoter activities, enhanced methylation of CpG and inhibited acetylation of histone 3 (H3) at the core promoter region of the LO gene. These results support the hypothesis that transcriptional and translational processes of LO are major targets for NNK. Thus, inactivation of the tumor suppressor gene LO may play a critical role in NNK carcinogenesis (supported by NIEHS 011340 and Philip Morris ERP)

PS 220 GT-094, A NO-NSAID, INHIBITS COLON CANCER CELL GROWTH BY ACTIVATION OF A REACTIVE OXYGEN SPECIES (ROS)-MICRORNA-27A:ZBTB10-SPECIFICITY PROTEIN (SP) PATHWAY.

S. Pathi[1] and S. Safe[1, 2]. [1]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX and* [2]*Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX.*

Ethyl 2-((2,3-bis(nitrooxy)propyl)disulfanyl)benzoate (GT-094) is a novel NO chimera containing an NSAID and NO moieties and also a disulfide pharmacophore that in itself exhibits cancer chemopreventive activity. In this study, the effects and mechanism of action of GT-094 were investigated in RKO and SW480 colon cancer cells. GT-094 inhibited cell proliferation and induced apoptosis in both cell lines and this was accompanied by decreased mitochondrial membrane potential (MMP) and induction of reactive oxygen species (ROS), and these responses were reversed after cotreatment with the antioxidant glutathione. GT-094 also downregulated genes associated with cell growth [cyclin D1, hepatocyte growth factor receptor (c-Met), epidermal growth factor receptor (EGFR)], survival (bcl-2, survivin), and angiogenesis [vascular endothelial growth factor (VEGF) and its receptors (VEGFR1 and VEGFR2)]. Results of previous RNA interference studies in this laboratory has shown that these genes are regulated, in part, by specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 that are overexpressed in colon and other cancer cell lines and not surprisingly, GT-094 also decreased Sp1, Sp3 and Sp4 in colon cancer cells. GT-094-mediated repression of Sp and Sp-regulated gene products was due to downregulation of microRNA-27a (miR-27a) and induction of ZBTB10, an Sp repressor that is regulated by miR-27a in colon cancer cells. Moreover, the effects of GT-094 on Sp1, Sp3, Sp4, miR-27a and ZBTB10 were also inhibited by glutathione suggesting that the anticancer activity of GT-094 in colon cancer cells is due, in part, to activation of an ROS-miR-27a:ZBTB10-Sp transcription factor pathway.

PS 221 MOLECULAR MECHANISM OF ACTION OF INSULIN SENSITIZING DRUG, METFORMIN, AS ANTICANCER AGENT IN PANCREATIC CANCER.

V. Vasanthakumari[1] and S. Safe[1, 2]. [1]*Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX and* [2]*Institute of Biosciences and Technology, Houston, TX.*

The biguanide metformin is one of the most widely used insulin-sensitizing drugs (ISDs) for treatment of type 2 diabetes mellitus. In addition to its antidiabetic effect there is increasing evidence that metformin inhibits cancer cell proliferation and induces cell cycle arrest. However the molecular mechanisms of the anticancer activities of metformin are poorly understood. In the present study we evaluated the antineoplastic effects of metformin in pancreatic cancer cells. The effects of metformin on inhibition of cancer cell growth was demonstrated by cell proliferation assays; western blot analysis and Real Time PCR were performed to determine changes in expression of genes associated with the tumorigenicity of pancreatic cancer. In Panc28 cells, metformin treatment resulted in a dose dependent inhibition of cell proliferation. The specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 that are usually overexpressed in pancreatic and other cancer cells were downregulated by metformin and this response was dose dependent. Cotreatement of pancreatic cancer cells with lactacystin (a compound which inhibits proteosome activity) and metformin, reversed the downregulation of Sp proteins. Furthermore, metformin-induced repression of Sp proteins was also reversed in Panc28 cells after cotreatment with sodium orthovanadate - a phosphatase inhibitor which specifically blocks tyrosine phosphatases. Treatment of Panc28 cells with metformin also decreased expression of Sp-regulated genes involved in tumorigenesis and these include cyclin D1(cancer cell growth), VEGF, VEGFR1(angiogenesis), survivin and bcl2(survival). The mechanisms of metformin induced phosphatase/ proteosome-mediated downregulation of Sp proteins and Sp-regulated genes are currently being evaluated.

PS 222 PHARMACOLOGICAL INHIBITION OF THE TGFβ1 TYPE I RECEPTOR INDUCES PREMALIGNANT KERATINOCYTE TERMINAL DIFFERENTIATION.

K. E. Masiuk, L. Mordasky Markell, N. Blazanin and A. B. Glick. *Center for Molecular Toxicology and Carcinogenesis, Penn State University, University Park, PA.*

The Transforming Growth Factor β1 (TGFβ1) pathway is important in the regulation of tumor suppression and promotion in skin cancer. To target this pathway, pharmaceutical companies have developed small molecule inhibitors of the TGFβ1 type 1 receptor (ALK5) kinase such as SB431542 (SB) that are potent in blocking the TGFβ1 signaling pathway. Our recently published data show that SB inhibits papilloma formation but enhances malignant conversion in a mouse multi-stage carcinogenesis assay. To test if SB inhibits tumor development through altered terminal differentiation (TD), we used a *RAS*-oncogene induced neoplastic keratinocyte model. SB enhanced *RAS*-induced cornification which correlated with the increased expression of TD genes transglutaminase 1 (*TGM1*) and 3 (*TGM3*) and small proline-rich protein 1A (*SPR1A*) and 2H (*SPR2H*) which crosslink structural proteins of the cornified envelope. There was a similar increase in cornified layers and *TGM*s following SB treatment of mice expressing inducible epidermal *RAS*. Alternatively, treatment of *RAS*-expressing keratinocytes with TGFβ1 or overexpression of *TGFβ1* and *RAS* in an inducible epidermal model resulted in reduced expression of *TGM*s and cornification *in vitro*. Papilloma (SP1) and squamous cell carcinoma (PAM2.12) cell lines were less responsive to TGFβ1 suppression of markers and marker induction by SB. Interestingly, a subset of *RAS*-expressing, SB-treated keratinocytes were resistant to TD and overcame senescence and rapidly immortalized, suggesting that they represent a progressed phenotype. However, these cells were unable to grow in elevated calcium or produce tumors after grafting nude mice indicating that they were not fully malignant. Thus, selective responsiveness to TD may represent a mechanism by which blocked TGFβ1 signaling can inhibit the outgrowth of preneoplastic lesions but may cause a more progressed phenotype in a separate keratinocyte population.

PS 223 PHENOBARBITAL-LIKE MODE OF ACTION FOR LIVER TUMORS IN CD1 MICE AND F344 RATS EXPOSED TO A NEW DEVELOPMENTAL COMPOUND (X11422208).

D. Geter[1], L. H. Kan[1], A. Wood[1], M. J. LeBaron[1], B. B. Gollapudi[1], J. Murray[1], C. Terry[3], R. Rasoulpour[1], C. Elcombe[2], A. Vardy[2], J. Ross[2] and R. Billington[3]. [1]*The Dow Chemical Company, Midland, MI,* [2]*CXR BioSciences, Dundee, United Kingdom and* [3]*Dow AgroSciences, Abingdon, Oxfordshire, United Kingdom.*

D. Geter1, H.L. Kan1, A. Wood1 M. LeBaron1, B. Gollapudi1, J. Murray1, C. Terry3, R. Rasoulpour1, C. Elcombe2, A. Vardy2, J. Ross2, R. Billington3. 1. The Dow Chemical Company, Midland, Michigan, USA, 48674. 2. CXR Biosciences, Dundee, Scotland, UK. 3. Dow AgroSciences, Abingdon, Oxfordshire, UK. In repeat dose rodent toxicity studies, a new developmental compound (X11422208) induced liver hypertrophy in mice and rats during the first week of exposure. In guideline carcinogenicity studies it produced liver tumors in CD1 mice at the high dose of 79.6 or 176 mg/kg/day, and in F344 rats at the high dose of 21.3 mg/kg/day. A series of mode-of-action (MoA) studies was undertaken to determine if the liver effects were mediated by nuclear receptor activation. Several receptors known to be involved in liver enlargement were investigated: AhR, CAR, PXR, and PPARα. In both the mouse and rat, transcriptional studies showed elevations in CAR, and to a lesser extent PXR, but not AhR or PPARα mediated gene responses. Activation of CAR and PXR were further confirmed by increases in PROD and BROD enzyme activity. BrdU and Ki-67 investigations showed a large increase in hepatocellular proliferation. Additional studies using a transgenic mouse that contained human receptors for PXR and CAR (hPXR/hCAR) and a second mouse model that was null for both receptors (PXRKO/CARKO) were conducted. In the PXRKO/CARKO mice, X11422208 did not induce liver changes demonstrating that activation of these receptors was required to elicit a liver response. X11422208 exposed hPXR/hCAR mice developed slight liver hypertrophy and CAR and PXR related genes and enzyme activity were elevated; however, hepatocellular proliferation was not observed in these humanized mice. It is concluded that X11422208 induces hepatocellular tumors in both the mouse and rat via a CAR-mediated MoA similar to phenobarbital and thus, would not induce tumors in humans.

PS 224 NON-HUMAN RELEVANCE OF A CAR-MEDIATED TUMOR FORMATION IN RATS INDUCED BY THE HERBICIDE METAZACHLOR.

C. Wiemann[1], N. Honarvar[1], M. Göttel[1], S. Gröters[2], E. Fabian[2], B. van Ravenzwaay[2] and I. Fegert[1]. [1]*Regulatory Toxicology Pesticides, BASF SE, Ludwigshafen, Germany and* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany.* Sponsor: R. Landsiedel.

Assessment of human relevance of liver tumors seen in rodents can be performed according to the WHO/IPCS proposal. An analysis was performed on the slightly increased incidence of benign liver tumors in female Wistar rats induced by metazachlor, a herbicide that did not give indication of genotoxic effects. In order to address the suggested Mode of Action (MoA) via the activation of the Constitutive Androstane Receptor (CAR) a series of mechanistic studies were performed also excluding other possible mechanisms. A Metazachlor-induced CAR-mediated MOA similar to that of phenobarbital was confirmed by showing 1) the induction of CAR-regulated Cytochrome P450 isoenzymes (CYP) of the CYP 2B family on mRNA and activity level, 2) nuclear accumulation of CAR on protein level 3) CAR-dependent activation of a phenobarbital responsive enhancer module-specific CYP 2B promotor region in transfected primary hepatocytes. Centrilobular hypertrophy and liver cell proliferation, considered as further characteristic key events for this MoA, were also observed in the Metazachlor-induced liver tissue. An Arylhydrocarbon Receptor-mediated mechanism was excluded by the absence of the respective mRNA and enzyme activity of the CYP 1A family (7-Ethoxyresorufin-O-deethylase). A peroxisome proliferator activated receptor α-mediated mechanism was excluded by lacking mRNA (CYP 4A1) and enzyme activity (CN- -insensitive palmitoyl CoA oxidation and lauric acid 12-hydroxylation) induction. The involvement of sustained cytotoxicity as a MoA was excluded since no early onset of inflammation and no necrosis in the liver were identified after treatment with metazachlor. The issue of non-relevance to humans was investigated in vitro in primary hepatocytes as well as in a transgenic mouse model expressing solely the human PXR/CAR genes. In conclusion, this database supports the exclusive association of the Metazachlor hepatotumorigenicity in the rat with CAR-mediated phenobarbital-like mechanisms.

PS 225 LIGAND-DEPENDANT ACTIVATION AND DEACTIVATION OF NUCLEAR RECEPTOR SUBFAMILY 4 MEMBERS IN PANCREATIC CANCER CELLS.

X. Li[1] and S. Safe[2, 3]. [1]*College of Medicine, Texas A&M Health Science Center, College Station, TX,* [2]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX and* [3]*Institute of Biosciences & Technology, Texas A&M Health Science Center, Houston, TX.*

Nuclear receptor related 1 (Nurr1) and nerve growth factor IB (Nur77 or TR3) are members of the nuclear receptor subfamily 4. Expression levels and modulation of activities of these transcription factors induces apoptosis in various cancer cell lines. Therefore these receptors are potential drug targets for clinical treatment of tumors. A group of methylene-substituted diindolylmethanes (C-DIMs) were screened for ligand-dependent activation and deactivation of Nurr1 and TR3 in Panc1 and Panc28 pancreatic cancer cell lines. Significant induction of Nurr1 activity was observed for compounds such as 1,1-bis(3'-indolyl)-1-(p-trifluoromethylphenyl)-methane (DIM-C-pPhCF3) and 1,1-bis(3'-indolyl)-1-(p-bromophenyl)methane (DIM-C-pPhBr). Increased and decreased TR3 dependent luciferase activities were also observed after treatment with 1,1-bis(3'-indolyl)-1-(p-benzophenyl)-methane (DIM-C-pPhC6H5) and 1,1-bis(3'-indolyl)-1-(p-hydroxyphenyl)-methane (DIM-C-pPhOH) respectively. These preliminary results were further examined by using response element sequences linked to a luciferase reporter gene. The study has identified, for the first time, several C-DIMs which activate or deactivate Nurr1 and TR3. Their effects on gene expression and inhibition of cancer cell growth are currently being investigated.

PS 226 CANNABINOIDS INDUCE TYROSINE PHOSPHATASES AND INHIBIT SP TRANSCRIPTION FACTORS IN COLON AND PROSTATE CANCER CELLS.

S. Sreevalsan[1], N. Kaminski[3] and S. Safe[1, 2]. [1]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX,* [2]*Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX and* [3]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI.*

Cannabinoids are being developed for cancer chemotherapy however the mechanisms associated with their antineoplastic activity are unclear. In this study we examined the effects of two agents that activate both CB1 and CB2 receptors, namely WIN 55,212-2 (WIN), a synthetic aminoalkylindole derivative and cannabidiol (CBD), a major constituent in the plant Cannabis sativa. Both CBD and WIN inhibited growth of SW480 colon cancer cells with IC50 values of 5.54 and 9.01 uM respectively and co-treatment of 15 uM CBD with CB1 and CB2 receptor antagonists AM251 and AM630 respectively blocked the growth inhibitory effects of CBD. In contrast, neither CB receptor antagonists inhibited the effects of WIN demonstrating that the anti-proliferative activity of CBD and WIN were receptor-dependent and independent respectively. The effects of WIN and CBD on expression of Sp transcription factors, Sp regulated genes and kinases were also investigated. WIN and CBD downregulated p-Akt expression and CBD also inhibited p-MAPK expression suggesting that WIN/CBD, like other phyto-derived-anticancer drugs may induce phosphatase activity. Treatment of SW480 cells with 15 uM CBD or 7.5 uM WIN induce expression of MKP-1, MKP-5, s-PAcP, c-PAcP and SHP-1 but not PTEN, PP2A, PTP1B, MKP-2 and DEP-1. Moreover phosphatase inhibition with sodium orthovanadate indicates that CBs primarily induce tyrosine phosphatases that have previously been characterized for anticancer activities and the role of CB-induced phosphatases on pro-carcinogenic pathways/genes is being investigated.

PS 227 HOPS (HUMULUS LUPULUS) INHIBITS ESTROGEN METABOLISM IN NON-TUMORIGENIC HUMAN BREAST EPITHELIAL CELLS (MCF-10A CELLS).

L. P. Hemachandra, R. P. Chandrasena, B. M. Dietz, S. Chen, G. F. Pauli, N. R. Farnsworth, G. R. Thatcher and J. L. Bolton. *Medicinal Chemistry & Pharmacognosy, University of Illinois at Chicago, Chicago, IL.*

Long-term exposure to estrogen increases the risk of hormone dependent cancers in women. Estrogen induced cell proliferation in estrogen receptor positive cells (hormonal pathway), and the formation of reactive estrogen o-quinones mediated by cytochrome P450s (chemical pathway) are believed to contribute to the estrogen carcinogenesis mechanism. Estrogens are oxidized by P450 1A1/1A2 to the catechol metabolites, 2-hydroxyestradiol, and by P450 1B1 to 4- hydroxyestradiol. Both catechols are further oxidized to form electrophilic o-quinones that can react with DNA and proteins. For years, plant extracts have been used worldwide, as dietary supplements for women's health particularly for menopausal symptom relief. Mounting evidence suggests that botanical dietary supplements have chemopreventive properties which could specifically protect women from the carcinogenic effects of endogenous estrogens through modulation of both hormonal and chemical mechanisms. The current study focuses on elucidating the influence of these extensively utilized botanicals on estrogen metabolism in normal breast epithelial cells (MCF-10A). MCF-10A cells are estrogen receptor negative non-tumorigenic human breast epithelial cells, which can be transformed into a malignant phenotype with estradiol. After treatment of MCF-10A cells with estradiol and botanical extracts, estrogen metabolites were analyzed using a sensitive LC-MS/MS method. Among the botanical extracts tested, hops (*Humulus lupulus*) showed a significant reduction in catechol estrogen formation whereas black cohosh (*Cimicifuga racemosa*) had little effect. These data suggest that the hop extract might possess chemopreventive activity through inhibition of genotoxic estrogen quinone formation in addition to its known antioxidant and induction of detoxification enzyme capabilities.

PS 228 ENDOCRINE TOXICANTS PROMOTE RESISTANCE TO ANOIKIS AND INVASIVENESS OF BREAST CANCER CELLS *IN VITRO* .

W. G. Foster and H. L. Cameron. *Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada.*

Epidemiological studies suggest that environmental toxicants (ETs) can enhance breast cancer tumour aggressiveness and decrease survival in women with the highest exposures. We hypothesize that ETs can promote breast cancer progression by increasing breast cancer cell survival and invasiveness. The effect of increasing concentrations of two high priority and unregulated ETs, bisphenol A (BPA) and dibutyl phthalate (DBP), a phthalate used in cosmetics and enteric-coated slow-release capsules, on characteristics of proliferation, the invasive and metastatic potential of breast cancer cells *in vitro*. The human breast cancer cell line MDA-MB-231 was cultured and treated with BPA, DBP or its major metabolite monobutyl phthalate (MBP) for 1–9 days. Proliferation rate was measured by BrdU incorporation and subsequent immunochemical detection by spectrophotometer. Viability was measured as reductive capacity of the tetrazolium salt MTS to its formazan product and quantified by spectrophotometry. Invasiveness was measured by seeding cells in wells coated with extracellular matrix. Cells that degraded and penetrated into the lower compartment were then quantified by fluorescent labelling and fluorescent spectrophotometry. Resistance to anoikis was measured by seeding cells in ultra low binding plates and quantifying the number of viable cells at day 9. None of the ETs affected proliferation or viability at any tested concentration. Bisphenol A increased

invasiveness at concentrations representative of human exposure (10^{-7}M) but had no effect on resistance to anoikis. DBP had no effect on invasiveness or resistance to anoikis while its major metabolite increased resistance to anoikis at concentrations representative of human exposure (10^{-8}M). Therefore, at concentrations relevant to human exposure, endocrine toxicants may promote breast cancer progression and metastasis by altering resistance to anoikis and invasiveness.

PS 229 ENVIRONMENTAL CHEMICALS AND BREAST CANCER—GEOGRAPHIC ASSOCIATION BETWEEN LIVESTOCK WASTE, CONTAMINATED SURFACE WATER, AND BREAST CANCER INCIDENCE IN THE SAN FRANCISCO BAY AREA.

H. Larson[1], M. Mun[1], W. Onyenwe[1] and D. E. Johnson[1, 2]. [1]*Nutritional Science & Toxicology, University of California Berkeley, Berkeley, CA and* [2]*California Breast Cancer & Chemicals Policy Project, San Francisco, CA.*

The aim of this study was to introduce a novel approach to understanding the high incidence of breast cancer in the San Francisco Bay area and, in particular, Marin County, which has the highest incidence. Geographical and environmental pollution data were compared with breast cancer statistics in order to produce a "geomedical" image of the disease and possible association with environmental contaminants. Technologies and information sources included ArcGIS9, ArcMap, NCI State Cancer Profiles, Scorecard, and Census of Agriculture Profiles by county. A strong association occurred between breast cancer incidence and animal waste pollution from livestock, making this the primary comparative focus. Accordingly, livestock waste pollution and breast cancer incidence rates were compared among eight counties in the California Bay Area: Marin, Alameda, Contra Costa, Napa, San Francisco, San Mateo, Santa Clara, Solano, and Sonoma. Two non-California counties (King County, WA; Delaware County, OH) were also selected for analysis based on population demographics, water sources, and potential surface contamination from livestock waste. The results demonstrate a visible correlation between high amounts of animal waste and high incidence rates of breast cancer. The definitive causal link has yet to be identified, but one possible explanation is that improperly stored waste from large, industrial livestock farms may be contaminating surface water sources. Farm animals are typically treated with hormones and antibiotics to promote growth, but these treatments also increase the amount of hormonal waste excreted. In Marin County, surface water is the primary source of drinking water; however, adequate well-controlled studies on hormone contamination and persistence in the supply do not exist.

PS 230 NOVEL P450 REDUCTASE KNOCKOUT MOUSE MODELS FOR STUDYING CHEMICAL CARCINOGENESIS IN THE MAMMARY GLAND.

Y. Yao[1], S. Liu[1], W. Yang[1], B. Moorthy[2], X. Ding[1] and J. Gu[1]. [1]*Wadsworth Center, New York State Department of Health, and School of Public Health, State University of New York at Albany, Albany, NY and* [2]*Baylor College of Medicine, Houston, TX.*

Microsomal P450 (P450) enzymes play a critical role in the metabolic activation of chemical procarcinogens. However, it has been difficult to determine whether, following systemic exposure to a given procarcinogen (such as 7,12-dimethylbenz[a]anthracene, DMBA), the bulk of the activated carcinogen, or its DNA adducts, in the breast tissue is derived from local metabolism or from the liver. The aim of this study is to test the hypothesis that target-tissue P450-mediated metabolic activation plays an important role in DMBA-induced formation of DNA adducts in the mammary gland (MG). This hypothesis is tested in mouse models, by modulating P450 activities in the liver, in all extrahepatic tissues, or in the MG alone, through conditional deletion (or suppression) of the gene for P450 reductase (Cpr), an enzyme required for the activity of all microsomal P450 enzymes. Initial studies on the liver-specific Cpr-null mouse indicated that hepatic P450 enzymes do not contribute significantly to DMBA-induced DNA-adduct formation in the MG, as determined by ^{32}P-postlabeling. For a more direct determination of the role of MG P450 enzymes in DNA-adduct formation, we have also been studying an extrahepatic-Cpr-low mouse, which has normal P450 activities in the liver, but much suppressed P450 activities in all extrahepatic tissues. The level of CPR protein in MG microsomes from the extrahepatic-Cpr-low mouse was found to be ~10% of the levels found in wild-type mice. In addition, a MG-Cpr-null mouse model, expected to have tissue-specific suppression of P450 activities in the MG, is also being prepared, by crossbreeding Cpr-lox mouse with the MMTV-Cre mouse. The extrahepatic-Cpr-low mouse and the MG-Cpr-null mouse will be studied for determinations of the contributions of target tissue metabolic activation to DMBA-induced DNA adduct formation in the MG. (Supported in part by NIH grant ES018884)

PS 231 IDENTIFYING INHIBITION PATHWAYS OF MAMMARY CANCER STEM CELLS BY CHEMICAL GENETICS.

D. Castro, J. Maurer and R. Oshima. *Tumor Development Program, Sanford-Burnham Medical Research Institute, La Jolla, CA.*

Studies have identified subpopulations of cells within tumors that drive tumorigenesis, termed cancer stem cells (CSCs). CSCs have also displayed increased resistance to traditional therapies. This persistent viability permits the regeneration of the tumor and contributes to the failure of chemotherapeutics. We have isolated mammary cancer stem cells from the mouse mammary tumor virus (MMTV)-Wnt-1 transgenic model of breast cancer. These tumorigenic progenitor cells express markers of both luminal (keratin 8) and basal cells (keratin 14) as well as stem cell associated markers for CD24, CD29, and CD49f. Cell cultures enriched for the bipotent progenitor ($K8^+$,$K14^+$) have generated tumors with the differentiated morphology of the tumor of origin from as few as a single cell. We employed a small library of 243 annotated kinase inhibitors in an effort to establish a chemical sensitivity profile towards the CSCs. Our initial screen with a dose of 1 μM identified 35 compounds with the ability to reduce cell survival by >50% after a 3 day incubation period. A cluster of 7 annotated Protein kinase C (PKC) inhibitors exhibited the most dramatic effects on cell survival. The hydroxylated form of staurosporine, (UCN-01), a derived anti-cancer agent, suppressed growth at submicromolar concentrations and produced an IC_{50} of 47 nM. The concerted effectiveness of the PKC inhibitors at low nanomolar concentrations indicate an inhibitory role for PKC and its downstream targets. In order to demonstrate selective toxicity for our CSCs, we are currently evaluating the effectiveness of validated targets on representative mammary cancer cell lines. We also plan to implement this screening method with a small library of the most current FDA-approved anti-cancer drugs. The chemical sensitivity profile of our CSCs will eventually be applied to human cancer cells to identify those that may be regulated in a similar way.

PS 232 SEVERE URINARY BLADDER CYTOTOXICITY AND REGENERATIVE HYPERPLASIA WITH DOSE RESPONSE INDUCED BY ARSENITE IN ARSENIC (+3 OXIDATION STATE) METHYLTRANSFERASE KNOCKOUT MICE.

M. Yokohira[2, 1], L. L. Arnold[2], K. L. Pennington[2], S. Suzuki[2], S. Kakiuchi-Kiyota[2], K. Herbin-Davis[3], D. J. Thomas[3], K. Imaida[1] and S. M. Cohen[2]. [1]*Oncopathology, Kagawa University, Faculty of Medicine, Kita-gun, Kagawa, Japan,* [2]*Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE and* [3]*Pharmacokinetics Branch, Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. EPA, Omaha, NE.*

Arsenic (+3 oxidation state) methyltransferase (As3mt) catalyzes reactions converting inorganic arsenic to methylated metabolites. We evaluated whether the As3mt null genotype in mice modifies cytotoxic and proliferative effects in urinary bladders of wild type mice after inorganic arsenic exposure. Previously we showed female As3mt KO mice treated with diet containing 0, 100 or 150 ppm arsenic as arsenite (AsIII) had systemic toxicity and significant effects on the urothelium. In the present study, we determined if the cytotoxic and proliferative effects of AsIII on the urothelium are dose dependent over 4 weeks of treatment. Female wild type C57BL/6 mice and As3mt KO mice were divided into 5 groups each (n=7) with free access to drinking water containing 0, 1, 10, 25 or 50 ppm arsenic as AsIII for 4 weeks. At sacrifice, urinary bladders of both As3mt KO and wild type mice showed hyperplasia by light microscopy. In As3mt KO mice, numerous cytoplasmic and nuclear granules were present in all urothelial layers and were more abundant and larger than in wild type mice. In As3mt KO mice, livers showed mild acute inflammation and kidneys showed hydronephrosis. Thus, diminished arsenic methylation in As3mt KO mice exacerbates the effects of AsIII on the bladder epithelium, showing that altered kinetic and dynamic behavior of arsenic can affect its systemic and urothelial toxicity.

PS 233 TUMORS AND PROLIFERATIVE LESIONS IN ADULT OFFSPRING AFTER MATERNAL EXPOSURE TO METHYLARSONOUS ACID DURING GESTATION IN CD1 MICE.

M. Waalkes[2, 1], B. A. Diwan[3], E. J. Tokar[2, 1] and D. J. Thomas[4]. [1]*NCI at NIEHS, Research Triangle Park, NC,* [2]*NTP, NIEHS, Research Triangle Park, NC,* [3]*IRSP, SAIC, NCI, Frederick, MD and* [4]*U.S. EPA, Research Triangle Park, NC.*

Inorganic arsenic exposure is carcinogenic in humans and rodents. When pregnant mice are exposed to inorganic arsenic in the drinking water their offspring, when adults, develop tumors and proliferative lesions at several sites, such as lung, liver,

adrenal, uterus, ovary and oviduct. There is some suspicion that biomethylation products of inorganic arsenic, such as methylarsonous acid (MMA3+), may be an important carcinogenic species. Thus, we exposed pregnant CD1 mice to MMA3+ at 0 (control), 12.5 and 25 parts per million (ppm) in the drinking water from gestation day 8 to 18, stopped exposure and assessed tumors and proliferative lesions in groups (initial n = 25) of male and female offspring until two years of age. These doses did not impact survival or body weights. Female offspring showed significant increases in total epithelial ovarian tumors (primarily adenoma; control 0%; 12.5 ppm 39%; 25 ppm 26%), adrenal cortical adenoma at 25 ppm (control 0%; 25 ppm 26%) and epithelial uterine tumors at 12.5 ppm (primarily carcinoma; control 0%; 12.5 ppm 22%). Females also showed dose-related increases in oviduct hyperplasia (control 4%; 12.5 ppm 35%; 25 ppm 43%). Male offspring showed an increase in hepatocellular carcinoma at the highest dose (control 0%; 25 ppm 21%), and at the lower dose increased adrenal adenomas (control 0%; 12.5 ppm 28%) and lung adenocarcinoma (control 17%; 12.5 ppm 44%). Male offspring from MMA3+-treated mothers showed some unusual testicular tumors and lesions including 2 rete testis carcinomas, 2 rete testis adenomas, 4 rete testis hyperplasias, 3 testicular interstitial cell (IC) tumors, and 2 testicular IC hyperplasias. Overall, gestational maternal exposure to MMA3+ produces some of the same tumors as similarly applied inorganic arsenic, although the testicular lesions seen in the present work with MMA3+ were not previously observed with inorganic arsenic. (This abstract does not reflect US EPA policy).

PS 234 ACTIVATION OF PPARα BY PHTHALATES IS A NECESSARY KEY EVENT IN THE MODE OF ACTION (MOA) LEADING TO INDUCTION OF RODENT LIVER TUMORS – A RE-EXAMINATION OF DATA AND DATA GAPS.

A. Bachman[1], R. David[2], J. Butala[3] and J. Deyo[4]. [1]*ExxonMobil Biomedical Sciences Inc., Annandale, NJ,* [2]*BASF Corporation, Florham Park, NJ,* [3]*Consultant for Ferro Corporation, Gibsonia, PA and* [4]*Eastman Chemical Company, Kingsport, TN.*

Phthalate esters, notably, DEHP and DINP, induce changes in rodent livers resulting in hepatocellular carcinomas following treatment with high doses. The evidence demonstrates that the MOA by which DEHP and DINP induce liver cancer in rodents is through the activation of PPARα, a mechanism not relevant for humans. However, recently this MOA as a sole explanation for rodent hepatocarcinogenesis has been questioned (Guyton et al., 2009). Data cited as supporting the need for re-evaluation include results of a 2 year bioassay in PPARα null mice which showed a slightly increased incidence of liver tumors following DEHP administration and data indicating no liver tumor formation in the LAP-VP16 mouse model where PPARα was constitutively activated in hepatocytes for 1 year. In response to the re-examination of the MOA for phthalate-induced liver tumorigenesis, experts were invited to discuss the following question: Is activation of PPARα necessary and sufficient for phthalate-induced rodent liver tumorigenesis? The recent data was discussed and a consensus was reached. Activation of PPARα is necessary for phthalate-induced rodent liver tumorigenesis although it alone may not be sufficient. Other modes of action were considered but were not applicable. Humans do not appear susceptible to peroxisomal proliferation (PP) as exemplified by clinical studies of populations exposed for long periods to hypolipidemic drugs (rodent hepatocarcinogens and strong rodent PPs). Liver tumors and the MOA (including recently proposed alternative MOAs) by which they form are likely not relevant to humans; however, a data gap for demonstrating the lack of cell proliferative events (key event in the rodent MOA) in humans was acknowledged. The thoughts and comments of these deliberations are presented.

PS 235 TOXICOLOGY STUDY OF SENNA IN C3B6F1/N TRP53 HAPLOINSUFFICIENT MICE.

I. Surh[1], A. Brix[2], J. French[3] and J. Dunnick[1]. [1]*Toxicology Branch, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC,* [2]*Pathology Group, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC and* [3]*Host Susceptibility Branch, National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

Senna is a pod or leaf of Senna alexandrina P, Mill and is used as a stimulant laxative. In the large intestine, bacterial enzymes break the β-glycosidic bond of sennosides and release rhein-9-anthrone, the active form for the laxative effect. The laxative effect is driven by peristalsis and reduction of water and electrolyte absorption. To determine any potential toxic effects of senna, 5-week studies in the C57BL/6N mouse and 40-week short- term toxicology and carcinogenesis studies in the C3B6F1/N Trp53 haploinsufficient (p53(+/-)) mouse were conducted. In the 5 week studies, C57BL/6N mice were exposed up to 10,000 ppm senna in feed. There were no differences in survival, feed consumption or body weights between exposed and control groups. Increased incidences of epithelial hyperplasia of the cecum and colon were observed in males and females exposed to 5,000 or 10,000 ppm senna. These intestinal lesions were not considered to be of sufficient severity to cause mortality and, thus, in the p53(+/–) mouse 40-week study, the high dose of 10,000 ppm was selected along with lower senna doses including one calculated to deliver a dose closer to human exposure levels. Mean body weights and feed consumption of exposed p53 (+/-) mice were generally similar to that of controls. Significant increases in the incidences of epithelial hyperplasia of the colon and cecum were observed only at 10,000 ppm in p53(+/-) males and females, and the incidence of hyperplasia of the colon was significantly increased at 3,000 ppm in females. Our senna study findings in mice are consistent with published studies which showed that senna increased the incidence of intestinal hyperplasia in Sprague-Dawley rats.

PS 236 GLOBAL DE-REGULATION OF GENE EXPRESSION, OVER-EXPRESSION OF MICROTUBULES/MICROFILAMENTS, AND ALTERED CA+2 GRADIENTS, IN NI-TRANSFORMED 10T1/2 MOUSE EMBRYO CELLS.

J. R. Landolph[1, 2, 3], A. T. Pehl-DeSilva[2, 3], D. Mai[1, 3], J. K. Lin[1] and J. Zheng[1]. [1]*Department of Molecular Microbiology/Immunology, Keck School of Medicine, University of of Southern California, Los Angeles, CA,* [2]*Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA and* [3]*Comprehensive Cancer Center, University of Southern California, Los Angeles, CA.*

Carcinogenic Ni3S2 and green NiO were phagocytosed into and induced morphological/A. I./neoplastic transformation in 10T1/2 mouse embryo cells. mRNA differential display showed 130 genes were differentially expressed between non-transformed and two MCA/four Ni-transformed, 10T1/2 cell lines. Ni/MCA-transformed cell lines showed ect-2 gene amplification/higher levels of ect-2 mRNA/protein and absence of DRIP80/β-centaurin-2 mRNAs. We hypothesized amplification of ect-2 leads to higher steady-state levels of rhoA-GTP, inducing higher steady-state levels of microtubules (MTs) in Ni/MCA transformed cell lines, and confirmed this by staining non-transformed/transformed 10T1/2 cell lines with fluorescent antibody to α-tubulin/β-tubulin. MTs were present at higher levels/aggregated in transformed cells. We next hypothesized transcriptional silencing of the β-centaurin-2 gene causes higher steady-state levels of microfilaments (MFs) and alters their intracellular distribution in transformed cell lines. We confirmed this by staining cells with fluorescent phalloidin and showed MFs were present at higher levels/-aggregated in transformed cells. Third, we hypothesized silencing DRIP80 gene altered Ca+2 ion gradients in transformed cell lines, and confirmed this by staining cells with Ca+2 fluorophore FLUO 3AM. This would alter activities of Ca+2-dependent enzymes in transformed cell lines and cellular physiology. Therefore, Ni+2 ions caused differential expression of 130 genes, overexpression of MTs/MFs, altered cell shapes, and aberrant Ca+2 ion gradients, contributing to induction/maintenance of transformed phenotypes. Support: R01 ES 03341 (PI, JRL), Core Grant 5P30 CA014089/NIH, and funds from M. S. Prog./Micro. and Provost's Office at USC.

PS 237 SKIN TUMOR INITIATION/PROMOTION EVALUATION OF OXIDIZED ASPHALT (BITUMEN) FUME CONDENSATE.

J. J. Freeman[1], C. A. Schreiner[2], E. W. Arp[3], D. M. Burnett[4], C. R. Clark[5], S. Mahagaokar[6], C. M. Parker[7], C. W. Stewart[8] and M. S. Swanson[7]. [1]*ExxonMobil Biomedical Sciences, Inc., Annandale, NJ,* [2]*C & C Consulting in Toxicology, Meadowbrook, PA,* [3]*Asphalt Institute, Lexington, KY,* [4]*BP Corporation, Naperville, IL,* [5]*ConocoPhillips, Bartlesville, OK,* [6]*Shell Oil Company, Houston, TX,* [7]*Marathon Petroleum, Findlay, OH and* [8]*MPI Research, Mattawan, MI.*

Asphalt (bitumen) fume condensate (sample TRA) was collected from the headspace above a Type III built up roofing asphalt tank and evaluated for skin tumor initiation or promotion activity in a ~6-month assay in male Crl:CD1® mice. TRA met pre-determined acceptance criteria that ensured its similarity to field-generated fumes. The sample was previously reported (SOT 2009) to have weak skin carcinogenic activity in C3H mice. Interpretation of the cancer bioassay was somewhat confounded because the sample had low but not zero mutagenic potential, but also elicited prolonged skin irritation, suggesting a possible role for tumor promotion via hyperplasia and proliferation. The initiation phase of this study was defined as weeks 1 and 2 with the promotion phase beginning on week 3. TRA was tested for both initiating and promoting activity. TRA was diluted to a 67% solution in mineral oil (MO) and applied dermally twice per week (37.5 µl/application) during the appropriate phases. Positive control substances were 7,12-dimethylbenz(a)an-

thracene (DMBA,50 μg in acetone, applied once) as an initiator and 12-O-tetradecanolyphorbol-13-acetate (TPA,5 μg in acetone, applied twice weekly) during the promotion phase. Clinically observed masses following treatments to 30 mice/group, reported as tumor bearing animals (TBA), were as follows: MO / MO: 0; DMBA / TPA: 29; DMBA/TRA: 2; TRA / TPA: 8; MO / TPA: 1. In the TRA / TPA group at study termination, 3 of the 8 previously identified masses on TBA were no longer evident and 5 TBA were histologically confirmed as having papillomas. These results suggest that TRA was a weak tumor initiator in this assay.

PS 238 TUMOR NECROSIS FACTOR-α IS INVOLVED IN INFLAMMATION-MEDIATED SYSTEMIC GENOTOXICITY.

A. M. Westbrook[1], B. Wei[2], K. Hacke[2], J. Braun[2] and R. H. Schiestl[1, 2]. [1]*Molecular Toxicology, University of California Los Angeles, Los Angeles, CA and* [2]*Pathology and Lab. Medicine, University of California Los Angeles, Los Angeles, CA.*

Tumor necrosis factor (TNF)-α is a proinflammatory cytokine highly upregulated and distributed in chronic inflammatory diseases including inflammatory bowel disease (IBD). In order to determine the sufficiency and necessity of TNF-α in mediating systemic DNA damage, as seen during intestinal inflammation, TNF-α was injected into wildtype mice, or administered ectopically in primary splenic T-cells. Necessity was determined by chemically inducing intestinal inflammation in TNF receptor (TNFR) 1/2 double knockout mice and determining resultant systemic genotoxicity. DNA damage was measured as strand breaks measured by the alkaline comet assay and γH2AX immunostaining. TNF-α was sufficient to induce DNA damage in wildtype mice in the peripheral blood, mesenteric and peripheral lymph nodes. Levels of DNA damage were sustained for a longer period of time when combined with injection of interleukin (IL)-1β. These observations were also recapitulated in vitro to primary splenic T-cells, indicating the role of TNF signaling and the sufficiency of a single cell type to incur DNA damage. Co-incubation with CD11b+ monocytes resulted in further DNA damage. TNFR1/2 double knockout mice with chemically induced colitis incurred significantly less DNA damage, though not completely diminished, than wildtype mice with colitis, further supporting the role of TNF-α and TNF signaling in mediating inflammation-associated systemic DNA damage. TNF-α is therefore sufficient but not completely necessary to induce genotoxicity.

PS 239 IDENTIFYING ONCOMUTATIONS THAT WILL BE USEFUL QUANTITATIVE BIOMARKERS OF CARCINOGENIC EFFECT SUITABLE FOR SUB-CHRONIC TOXICITY TESTING.

M. B. Myers, Y. Wang, F. Meng, P. B. McKinzie, K. L. McKim and B. L. Parsons. *Division of Genetic and Molecular Toxicology, National Center for Toxicological Research/FDA, Jefferson, AR.*

We are developing specific hotspot oncogene mutation (oncomutations) as quantitative biomarkers of carcinogenic effect that can be incorporated into drug development as part of sub-chronic toxicity testing. The method being employed to quantify oncomutation is called allele-specific competitive blocker-PCR (ACB-PCR), which can reliably quantify base substitution mutations at frequencies as low as one mutant allele per 100,000 wild-type alleles. To identify which oncomutations will be the most useful biomarkers in various organs/tissues, we are systematically collecting data to fill three specific data gaps: 1) we are defining normal and pathological levels of various oncomutations in particular tissues, 2) determining which are the most prevalent oncomutations in particular tumor types, as measured by ACB-PCR, and 3) identifying oncomutations that have a similar profile in particular rodent tissues as in analogous human tissues. To date we have measured K*RAS* codon 12 GAT and GTT mutation in 60 normal human tissues, including colon, lung, pancreas, and thyroid. Tissue-specific K*RAS* codon 12 GAT mutant fractions (MF; given as the geometric mean) ranged between 9.46 x 10^{-6} for pancreas to 7.63 x 10^{-5} for colon. The GTT MFs ranged between 9.94 x 10^{-6} to 2.14 x 10^{-5} for colon and lung, respectively. Population distributions of tumor MFs in approximately 80 tumors that develop from these tissues generally include MF measurements higher than observed in normal tissues. For example, the thyroid tumor K*RAS* codon 12 GTT MFs were significantly higher than that of normal thyroid (6.27 x 10^{-4} versus 1.64 x 10^{-5}). An additional mutation being measured in these tissues and tumors is *PIK3CA* codon 1047 CAT to CGT mutation. By integrating human ACB-PCR oncomutation measurements with parallel measurements from rodents, it should be possible to establish the most appropriate biomarkers of carcinogenic effect in particular organs.

PS 240 AEROSOL DELIVERED-AKT KD ATTENUATED CLARA CELL INJURY INDUCED BY NAPHTHALENE.

A. Minai Tehrani and M. Cho. *Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea.*

Lung cancer has emerged as a leading cause of cancer death in the world; however, most of the conventional therapies are not effective in altering the progression of disease.therefore, new approaches such as gene therapy are needed for cancer treatment. Gene therapies for the treatment of lung cancer patients were delivered through several approaches but among them aerosol delivery is a potentially more productive approach for gene delivery, In this study Akt mutant (KD) and Akt wild type (WT) were delivered into the lungs of CMV-LucR-cMyc-IRES-LucF dual reporter mice through a nose–only inhalation system using (glucosylated PEI) GPEI and naphthalene was administrated to mice via i.p injection. Aerosol delivery of Akt WT and naphthalene treatment increased protein levels of downstream substrates of Akt signaling pathway while aerosol delivery of Akt KD did not, our results showed that naphthalene altered ERK protein level and caused Clara cell injury . However, the Clara cell injury induced by naphthalene was attenuated in mice exposed to Akt KD, in addition, dual luciferase activity assay showed that aerosol delivery of Akt WT and naphthalene treatment reduced cap-independent, while an increase in cap-independent protein translation by delivering Akt KD was observed. Our results suggest that the aerosol delivery is compatible for in vivo gene delivery which can be used as a noninvasive gene therapy

PS 241 ABCB1 PROTECTS KIDNEY PROXIMAL TUBULE CELLS AGAINST CADMIUM-INDUCED APOPTOSIS: ROLES OF CADMIUM TRANSPORT AND CERAMIDE METABOLISM.

W. Lee, B. Torchalski, N. Kohistani and F. Thévenod. *Physiology & Pathophysiology, University of Witten/Herdecke, Witten, NRW, Germany.*

Multidrug resistance P-glycoprotein (ABCB1) confers resistance to cancer therapy and enhances cell survival. Cadmium (Cd) damages the kidney proximal tubule (PT) and is a class I carcinogen. We have previously shown that Cd induced apoptosis involves ceramide formation (*Am J Physiol Cell Physiol* 293: C839, 2007) and that Cd upregulates ABCB1 to increase cell survival (*J Biol Chem* 275: 1887, 2000). Here, we challenge published data that ABCB1 can directly transport Cd as a mode of cellular protection. Classical ABCB1 inhibitors (PSC833, UIC-2) did not affect ^{109}Cd efflux in PT cells though ABCB1 transport function, measured by rhodamine 123^+ efflux, was blocked. Furthermore, ABCB1 overexpressing MDCK cells did not extrude more ^{109}Cd compared to controls. Increased ABCB1 expression, either by active Wnt signaling or permanent transfection (ABCB1-MDCK), attenuated apoptosis by 10-50 μM Cd or 5-25 μM C_6-ceramide that was reversed by PSC833 (1 μM). Measurements of ABCB1-MDCK cell monolayer integrity using ECIS (Applied Biophysics) exhibited similar effects with PSC833 and 25 μM Cd. Interestingly, Cd-induced ceramide formation, measured by a diacylglyercol kinase assay, was abolished in ABCB1-MDCK, suggesting expulsion of ceramide or one of its metabolites by ABCB1. Apoptosis by Cd was significantly decreased as a consequence of lowered (glucosyl)ceramide by 0.1 μM fumonisin B_1, a blocker of de novo ceramide synthesis, and 2 μM C_9DGJ, which inhibits ceramide to glucosylceramide conversion. In contrast, increased ceramide by 0.1 mM D609 and 1 μM dimethylsphingosine, inhibitors of sphingomyelin synthase and sphingosine kinase, respectively, augmented Cd-induced apoptosis. Hence, we demonstrate that ABCB1 protects PT cells from undergoing apoptosis but not by effluxing Cd. Rather, the sphingolipid (glucosyl)ceramide appears to be the toxic metabolite extruded by ABCB1 leading to cell survival that could be implicated in propagating Cd carcinogenesis.
Funded by DFG TH345/10-1.

PS 242 EVALUATION OF THE TUMORIGENIC POTENTIAL OF A MIXTURE OF FIVE PESTICIDES USING A MEDIUM-TERM HEPATOCARCINOGENESIS ASSAY WITH THE LEWIS RATS.

J. V. de Camargo, C. S. Franchi and M. F. Martinez. *Pathology, Sao Paulo State University - UNESP - Medical School, Botucatu, Brazil.* Sponsor: D. Wolf.

INTRODUCTION: In a country-wide survey, the pesticides dieldrin, dichlorvos, permethrin, dicofol and endosulfan were found by the Brazilian Agency for Health Surveillance (ANVISA) contaminating tomatoes regularly available over-the-counter for Brazilian consumers. The relevance of this exposure to human health is largely unknown. A medium-term bioassay was developed in order to explore the carcinogenic potential of a dietary mixture of these five pesticides on the liver of the

Lewis male rat. METHODS: pesticide levels in the diet were adjusted according to their respective NOEL (MIX1: Non-effective Levels) or LOAEL, LEL or LOEL (MIX2: Effective Levels), as referred in the literature. The carcinogenic potential on the liver was assessed by a 8-week long initiation-promotion assay which initiates liver carcinogenesis by diethylnitrosamine (DEN) and uses the quantitative development of the putative preneoplastic glutatione S-transferase positive (GST-P+) altered foci of hepatocytes as end-point marker. The adopted bioassay is based on the so-called Ito's bioassay, originally standardized with the Fischer 344 male rat. Along with other evidences, the Ito's protocol is recommended by the International Conference on Harmonization (ICH) as an acceptable alternative to the long-term rodent carcinogenicity test. RESULTS: Although numerically increased, the number and size of GST-P+ foci in DEN-MIX1 and DEN–MIX2 groups did not differ significantly from the DEN-initiated group, probably due to the large variability of foci number and size within groups. CONCLUSION: These data suggest that these mixtures of pesticides are weak rat hepatocarcinogens or liver tumor promoters. Therefore, using the ICH approach for evaluation, this study suggests that the mixtures evaluated should not pose a cancer hazard. SUPPORT: FAPESP 2006/60506-1.

PS 243 PRELIMINARY EVALUATION OF THE HUMAN RELEVANCE OF RESPIRATORY TUMORS OBSERVED IN RODENTS EXPOSED TO NAPHTHALENE.

V. Piccirillo[1], M. G. Bird[2], R. Lewis[2] and W. Bover[2]. *[1]VJP Consulting, Inc., Ashburn, VA and [2]ExxonMobil Biomedical Sciences, Inc., Annandale, NJ.*

Naphthalene rodent bioassays have shown significant dose-related increases in respiratory epithelial adenomas in male rats, olfactory epithelial neuroblastomas in female rats, and pulmonary bronchial adenomas in female mice. We describe a mode of action (MOA) / Human relevance (HR) framework for naphthalene. It sets out transparently, the weight of evidence for a hypothesized MOA for experimental animals and humans in the context of key events. It indicates a species difference in both carcinogenic and cytotoxic response between both the rodent and human based on qualitative and quantitative differences in metabolism. This occurs both at the initial oxidation of naphthalene due to the preferential formation of the cytotoxic diol and ortho naphthoquinone in rat and mouse through CYP2F4 and CYP2F2, respectively, versus naphthol formation and the para naphthoquinone via CYP2A13 metabolism, which is unique to the human respiratory system. In the human, this predominates over CYP2F1. Normally, subsequent reactive metabolites are conjugated through glutathione, but under high dose exposures resulting in glutathione depletion, it is likely that metabolism is re-directed to secondary, low affinity pathways. Quinone imines are associated with cancer in the rat nose and mouse lung for alachlor and phenacetin. In the rat nose, current research to assess subsequent formation of a naphthoquinone imine due to the presence of site-specific aryl amidase acting on an amino acid conjugate of the quinone will be described. This amidase is minimally present in the mouse and human nose but is significantly present in target mouse lung Clara cells. There is no human evidence of nasal or lung tumor risk for naphthalene, although the available human data have limitations. In conclusion, while further research is ongoing, the carcinogenic response observed in rodents does not appear relevant to the human based on MOA and the limited epidemiological data available.

PS 244 SMALL MOLECULE RAF INHIBITORS CAUSE EXTENSIVE MULTIPLE TISSUE HYPERPLASIA AND AN INCIDENCE OF URINARY BLADDER TRANSITIONAL CARCINOMA IN THE RAT WITHIN 28-DAYS.

J. Wisler[1], C. Afshari[1], M. Fielden[1], C. Zimmermann[1], S. Taylor[1], J. Carnahan[2] and S. Vonderfecht[1]. *[1]Comparative Biology Safety Sciences, Amgen, Thousand Oaks, CA and [2]Hematology & Oncology Research, Amgen, Thousand Oaks, CA.*

V600E mutant B-Raf is a clinically validated therapeutic target in melanoma patients. However, consistent with squamous cell carcinomas observed in patients treated with Raf inhibitors, we and others have found evidence that exposure of cells and tumors harboring wild type Raf to small molecule Raf inhibitors can result in sustained activation of MAPK signaling and tumor growth increase. Seven potent Raf kinase inhibitors (C1-7) were evaluated in 7-day oral rat toxicity studies. All compounds induced hyperplasia in multiple tissues. Consistently affected was stratified squamous epithelium at a number of sites and transitional epithelium of urinary bladder and kidney. Proliferative changes were also observed in heart and sciatic nerve. A 7-day time course study in rats showed evidence of epithelial proliferation (increased mitotic figures and number of cells staining for pHistone H3) in the nonglandular stomach within 4-5 hours after a single dose of C-1: a nongenotoxic molecule. Cellular proliferation was also observed in the urinary bladder by day 2 and in the heart, kidney and liver by day 3. Transcriptional evidence of proliferation in the bladder was detected within 4-5 hours after a single dose based on induction of genes in the PI3K/AKT and ERK/MAPK pathways. In a 28-day rat toxicity study of C-1, hyperplasia was observed in the esophagus, nonglandular stomach, skin, urinary bladder, kidney, and heart. Hyperplasia of transitional epithelium of the urinary bladder was particularly severe and transitional cell carcinoma was observed in one high dose female rat. These results suggest that these Raf inhibitors induce early transcriptional changes driving unchecked cell proliferation resulting in marked tissue hyperplasia within a short time frame. We propose that the rare appearance of a transitional cell urinary bladder carcinoma after 28-days was likely via a nongenotoxic mechanism.

PS 245 PATHWAY DIRECTED MOLECULAR ANALYSIS OF LARGE INTESTINAL TUMORS INDUCED IN FISHER 344 RATS EXPOSED TO ALOE VERA WHOLE LEAF EXTRACT IN DRINKING WATER.

A. R. Pandiri[1, 2], R. C. Sills[1], M. J. Hoenerhoff[1], T. T. Ton[1], H. L. Hong[1], G. P. Flake[1], D. E. Malarkey[1], I. Pogribny[4], N. J. Walker[3] and M. D. Boudreau[4]. *[1]Cellular and Molecular Pathology Branch, National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC, [2]Experimental Pathology Laboratories, Inc., Research Triangle Park, NC, [3]NTP/NIEHS, Research Triangle Park, NC and [4]National Center for Toxicological Research, Jefferson, AR.*

Aloe vera (AV) is one of the most commonly used botanicals for various prophylactic and therapeutic purposes. Aloe products contain variable amounts of polysaccharides like acemannans as well as mixtures of anthrones and anthraquinones (Boudreau and Beland, 2006). Recent NTP/NCTR chronic studies have demonstrated a dose dependent increase in large intestinal tumors in F344 rats exposed to Aloe vera whole leaf extract in drinking water (NTP TR# 577). In this study, we compared the morphological and molecular features of AV-induced large intestinal tumors in the F344 rats with those of human colorectal cancer. Based on defined histological criteria, 8/12 samples were diagnosed as colonic adenomas (CAd) and 4/12 were diagnosed as carcinomas (CCa) (Boivin et al., 2003). Mutation analysis of 8 CAds and 4 CCas identified point mutations in codons 12, 13 and 61 of the Kras gene (2/8 CAd, 2/4 CCa), and in codons 32, 34, 41 and 45 of the Ctnnb1 gene (3/8 CAd, 1/4 CCa). No Tp53 (exons 5-6 and 7-8) mutations were observed in CAds or CCas. Catalogued (MAPK, Wnt, TGF-β) and custom (designed from human colorectal cancer (hCRC) literature and meta-analysis of 10 hCRC microarray datasets) PCR arrays were used to examine the differential gene expression of CAd and CCa. Molecular pathways important in hCRC such as MAPK, Wnt and TGF-β signaling were over represented along with inflammatory pathways including NF-κB, IL-8 and glucocorticoid receptor signaling in AV-induced CAds and CCas in F344 rats. We conclude that the AV-induced CAds and CCas in F344 rats are very similar to hCRC at the morphological and molecular levels and that these results will aid in risk assessment.

PS 246 EFFECTS OF CHRONIC ACRYLAMIDE EXPOSURE ON SYSTEMIC HORMONAL ENVIRONMENT AND CARCINOGENIC TARGET ORGANS IN F344 FEMALE RATS.

T. Imai[1], T. Watari[1], T. Hayakawa[2] and T. Kitahashi[1]. *[1]Central Animal Laboratory, National Cancer Center Research Institute, Tokyo, Japan and [2]Cancer Prevention Basic Research Project, National Cancer Center Research Institute, Tokyo, Japan.*
Sponsor: A. Nishikawa.

Acrylamide (AA) has been reported to show genotoxicities in various *in vitro* and *in vivo* test systems and carcinogenicities in rats and mice. As target organs in rat long-term bioassays, some hormone-related organs, such as thyroid, adrenal, uterus and mammary gland are pointed out. Therefore, carcinogenic mechanisms associated with systemic hormonal dysregulation by AA exposure have been considered. However, so far supportive data on such mechanisms other than genotoxicities are limited. The objective of the present study is to clarify toxicological effects of chronic AA exposure on the systemic hormonal environment and the carcinogenic target organs in rats.
A total of 90 female F344 rats were divided into three groups and AA at 0 (control), 1.5 and 3.0 mg/kg body weight each was treated in drinking water for 52 and up to 104 weeks. At week 52, ten animals in each group were sacrificed under ether anesthesia after blood sampling. No significant change was observed in the levels of serum insulin, insulin-like growth factor-I, leptin and adiponectin, which are known as endogenous modifiers on mammary carcinogenesis and in the histopathology of thyroid, adrenal, mammary gland and uterus. Measurements for the levels of serum thyroid-related and sex hormones and for cell proliferative activities in the above mentioned organs are now on going. In addition, mammary tumor samples from the other twenty rats of each group will be collected, and it is design to analyze protein-expression profiles of signaling pathways in the tumors. At the annual meeting, these data would be comprehensively reported and discussed.

PS 247 CO-CARCINOGENESIS OF N, N-DIETHYLNITROSAMINE AND CARBON TETRACHLORIDE IN MOUSE LIVER.

G. Ainslie[1], T. Uehara[1], O. Kosyk[1], B. Bradford[1], G. Boorman[2] and I. Rusyn[1]. [1]*Department of Environmental Sciences and Engineering, University of North Carolina Chapel Hill, Chapel Hill, NC and* [2]*Covance, Princeton, NJ.*

Background: Liver fibrosis and cirrhosis are well known precursor liver disease states for hepatocellular carcinoma (HCC) in humans. In rodent 2-year bioassays, however, liver tumors arise on a normal liver which is one of the factors limiting their utility to predict human HCC. While several animal models have been used to examine the mechanisms involved in liver fibrosis, the promoting effect by fibrosis in the process of hepato-carcinogenesis is less understood. This study aimed to examine the mechanisms by which fibrosis is involved in tumor promotion in the liver. Methods: Male B6C3F1 mice were treated with a single dose (1 mg/kg) of N,N-diethylnitrosamine (DEN) or PBS at 15 days of age. Carbon tetrachloride (CCl4) treatment (0.2 ml/kg; i.p.; twice a week) was started at 8 weeks of age and continued for 9 or 14 weeks. Results: DEN-treatment alone led to a time-dependent increase in the incidence of hepatocellular altered foci (HAF, 80 and 95%). CCl4-treatment alone induced a small number of HAF and HCC only at the later time point (12.5 and 37.5%, respectively). CCl4-treatment after DEN-initiation had a significant effect at each time point on both incidence (40 and 100%) and multiplicity of neoplastic lesions in the liver. Severe liver fibrosis in CCl4-treated animals was confirmed by Masson's trichrome stain and an increase in TGFbeta expression. Cell proliferation, assessed by BrdU immunohistochemistry was dramatically elevated in all CCl4-treated groups. Liver inflammation was elevated concomitantly with the fibrotic changes and DNA repair genes were dys-regulated in both time points. Conclusions: This study provides strong evidence that progressive liver fibrosis is acting as tumor promoter after DEN-initiation, a mechanism which is highly relevant to the development of human HCC. Further studies with this model will enable evaluation of the mechanisms of human liver carcinogenesis.

PS 248 KNOCK DOWN OF AHR IN HUMAN BREAST CANCER CELL LINE UPREGULATES THE EXPRESSION OF PRO-APOPTOTIC FACTOR BAK.

G. Goode and S. E. Eltom. *Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN.*

AhR is overexpressed and constitutively activated in several human breast carcinoma cell lines and the expression levels showed strong correlation with the degree of the tumor malignancy. The objective of this study is to examine the effect of reducing the expression levels of AhR in metastatic human breast carcinoma (HBC) cell lines on their tumorigenic properties and the underlying molecular targets. We designed a series of short hairpin RNA (shRNA) targeting AhR and cloned them in pSuper retroviral vectors, and viruses expressing shRNA were used to infect HBC cell line MDA-MB231 to generate cell lines that permanently express siRNA targeting AhR. Clonal cell lines with 50-90% AhR knockdown (AhR-KD) were characterized in vitro for their tumorigenic properties in comparison to the scramble RNA-expressing control cell lines. RNA and protein extracts from these cell lines were compared for their gene and protein expression for markers responsible for modified phenotypes. AhR-KD cells exhibited prolonged doubling times and decreased cell proliferation. Cell cycle analysis by FACS showed a decreased transition of these cells from G1 to S and G2/M phases of cell cycle. Most significantly, the AhR-KD clones of MDA-MB231 cell line which is characterized by fibroblastic morphology have reverted to their epithelial morphology, indicating a role for AhR in regulating the epithelial to mesenchymal transition (EMT). Real time RT-PCR and Western blotting analyses reveals a dramatic upregulation of BAK a pro-apoptotic factor in AhR-KD cells, at mRNA and protein levels. These results demonstrate a major role for the AhR in regulating tumorigenic phenotypes of metastatic breast cancer and promoting cell survival by inhibiting apoptosis. Findings will establish the potential of AhR as a target for therapeutic intervention of metastatic breast cancer.

PS 249 A NOVEL SMALL MOLECULE INHIBITOR OF WNT/β-CATENIN SIGNAL PATHWAY INDUCES CELL CYCLE ARREST IN HUMAN NON-SMALL LUNG CANCER CELLS.

J. Choi[1], S. Lee[1], Y. Gong[2], Y. Park[1] and M. Dong[1]. [1]*School Lifesciences and Biotechnology, Korea University, Seoul, Republic of Korea and* [2]*Department of Chemistry, Dongguk University, Seoul, Republic of Korea.* Sponsor: B. Lee.

Aberrant activation of the Wnt/β-catenin signal pathway is linked to a high frequency of numerous tumors. In previous study, we screened the chemical library to develop the new drug for lung cancer therapy as a target of Wnt2/β-catenin pathway using a cell-based assay in A549/Wnt2 cells stably expressed wnt2. We chose one compound, KROR-759, which was reduced to below 50% of the luciferase activity at the cell proliferation IC50 concentration was taken. 759 reduced β-catenin-TCF/LEF dependent transcriptional activity in a dose-dependent manner with an IC50 value of about 2μM. To further address the action mechanism of KROR-759, we performed DNA microarray analysis by using Illumina BeadArray Technology, after treatment of A549 cells with 1uM KROR-759 for 24h. The data demonstrated that KROR-759 had a very selective effect on global gene transcription. KROR-759 was down-regulated the expressions of Wnt/β-catenin signaling pathway target genes, such as E2F, cyclin D1 and mini-chromosome maintenance (MCM), which play critical roles in cell growth, proliferation, and differentiation. KROR-759 induced accumulation of sub-G1 phase and arrests in the G1 phase of the cell cycle. Induction of G1 arrest by KROR-759 was correlated with inhibition of E2F, cyclin D1 and MCM expression. Moreover, mini-chromosome maintenance (MCM) proteins were markedly down-regulated in A549 cells treated with KROR-759. Collectively, these results suggested that KROR-759 possessed a potential anti-cancer activity against lung cancer cells by inhibiting their proliferation and inducing cell cycle G1 arrest.

PS 250 ARISTOLOCHIC ACID NEPHROPATHY: AN ENVIRONMENTAL AND IATROGENIC DISEASE.

A. Grollman[1], B. Jelakovic[2], Y. Pu[3] and C. Chen[3]. [1]*State University of New York at Stony Brook, Stony Brook, NY,* [2]*University of Zagreb, Zagreb, Croatia and* [3]*National Taiwan University, Taiwan, China.* Sponsor: J. Swenberg.

Aristolochic acid (AA), a principal component of Aristolochia species, was shown recently to be the toxin responsible for the clinical syndromes known as Chinese herb nephropathy and endemic (Balkan) nephropathy (EN). Both disorders are associated with a high incidence of upper urinary tract urothelial carcinomas (UUC) and appear to constitute a single disease entity, designated aristolochic acid nephropathy (AAN). Based on the traditional use of Aristolochia in herbal remedies, we posited that AAN represents a long-overlooked iatrogenic disease and an international public health problem of considerable magnitude (Adv Mol Tox 3, 211, 2010). Pursuing this hypothesis, we conducted studies of 100 patients with UUC residing in Taiwan, or in regions of Croatia, Bosnia and Serbia where EN is prevalent. DNA was obtained, with informed consent, from renal cortical and tumor tissues following nephroureterectomy. Aristolactam (Al)-DNA adducts in the renal cortex were quantified by 32P- post-labeling techniques. Chip-sequencing technology was utilized to establish the pattern of mutations in the TP-53 gene. Al-DNA adducts were detected in the majority of patients with UUC. The TP53 mutation spectrum was dominated by A:T→T:A transversions located almost exclusively on the non-transcribed DNA strand, reflecting a failure to excise AL-DNA adducts by global genomic nucleotide excision repair. This factor may account for the remarkable persistence of these adducts in human tissues. Thus, aristolochic acid joins aflatoxin and vinyl chloride as one the few human carcinogens with a definitive TP53 mutational signature. Coupled with the use of AL-DNA adducts as biomarkers, the presence of a mutational signature establishes an etiological role for AA in UUC. Public health authorities in countries where Aristolochia has been used are encouraged to initiate screening programs to detect UUC and to implement measures that will reduce human exposure to this nephrotoxic and carcinogenic herb. (Supported by NIEHS).

PS 251 VITAMIN E DEFICIENCY IN MICE TREATED WITH DICHLOROACETATE OR TRICHLOROACETATE MODULATES THE BIOMARKERS OF PHAGOCYTIC ACTIVATION AND ANTIOXIDANT ENZYME ACTIVITIES IN THE PERITONEAL LAVAGE CELLS.

E. Hassoun and A. Al-Dieri. *Pharmacology, Pharmacy, University of Toledo, Toledo, OH.*

Dichloroacetate (DCA) and trichloroacetate (TCA) are water chlorination by products. Previous studies in our lab have indicated induction of phagocytic activation in the peritoneal lavage cells of mice after subchronic exposure to the compounds. To study the effects of marginal vitamin E deficiency on DCA- and TCA-induced phagocytic activation, groups of mice were kept on either regular diet supplemented with vitamin E (E-normal group) or low vitamin E diet (E-deficient group). The two groups of mice were divided into subgroups and were treated daily, by gavage, with water (control groups), 77 mg DCA/kg/day and 77mg TCA/day for 13 weeks. Mice were killed at the end of the treatment period and peritoneal lavage cells (PLCs) were collected and assayed for biomarkers of phagocytic activation, including superoxide anion (SA) production and myeloperoxidase activity, and for activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX). DCA and TCA, in general, induced

significant increases in the various tested biomarkers in the E-deficient and E-normal groups, when compared to the corresponding controls in each of those groups. However, the DCA- and TCA-induced increases within the E-deficient subgroups were significantly greater than those of the corresponding subgroups within the E-normal group. Previous studies in our lab suggested phagocytic activation as a defense mechanism against DCA- and TCA-induced long-term hepatotoxicity. The present results, including phagocytic activation and antioxidant enzyme activities suggest up-regulation of those mechanisms in response to vitamin E deficiency to accommodate for the loss of protection by vitamin E against DCA and TCA long-term toxicities. (Supported by NIH/NIEHS grant # R15ES013706-01A2)

PS 252 XENOBIOTIC-INDUCED ENDOPLASMIC RETICULUM STRESS TRIGGERS PRO-INFLAMMATORY NF-κB SIGNALS *VIA* REPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ

Y. Moon and S. Park. *Microbiology and Immunology, Pusan National University School of Medicine and Medical Research Institute, Yangsan, Republic of Korea.*

Endoplasmic reticulum (ER) stress is a causative factor of human inflammatory bowel diseases (IBD). ER stress mediators including CCAAT-enhancer-binding protein (C/EBP) homologous protein (CHOP) are elevated in intestinal epithelia from patients with IBD. The present study arose from the question of how chemical ER stress and CHOP protein were associated with nuclear factor-κB (NF-κB)-mediated epithelial inflammatory response. In a human intestinal epithelial cell culture model, chemical ER stresses induced pro-inflammatory cytokine interleukin-8 (IL-8) expression and the nuclear translocation of CHOP protein. CHOP was positively involved in ER-activated IL-8 production and was negatively associated with expression of peroxisome proliferator-activated receptor γ (PPARγ). ER stress-induced IL-8 production was enhanced by NF-κB activation that was negatively regulated by PPARγ. Mechanistically, ER stress-induced CHOP suppressed PPARγ transcription by sequestering C/EBPβ and limiting availability of C/EBPβ binding to the PPARγ promoter. Due to the CHOP-mediated regulation of PPARγ action, ER stress can enhance pro-inflammatory NF-κB activation and maintain increased level of IL-8 production in human intestinal epithelial cells. In contrast, PPARγ was a counteracting regulator of gut inflammatory response through attenuation of NF-κB activation. The collective results support the view that balances between CHOP and PPARγ are crucial for epithelial homeostasis and disruption of these balances in mucosal ER stress can etiologically affect the progress of human IBD (This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2009-0087028)).

PS 253 PRO-INFLAMMATORY CYTOKINE AND COMPLEMENT RECEPTOR 3 (CR3) RESPONSES TO FUNGAL CELL WALL MANNAN AND MANNAN BINDING LECTIN (MBL) IN HUMAN WHOLE BLOOD AND MONOCYTES.

A. M. Nilsen[1], I. Rauk[1], M. Løvik[1, 2] and L. N. Johannessen[1]. [1]*Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway and* [2]*Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway.*

A correlation between fungal exposure and aggravation of respiratory symptoms in asthmatic individuals is well documented. In severe asthma, mannan binding lectin (MBL) has been found to modulate the inflammatory cytokine production. We have observed a reduced production of TNF-α by monocytes after exposure to *S. cerevisiae* cell wall mannan in adult humans with mild asthma, (Johannessen et al., 2008), suggesting a role for MBL even in mild disease. The hypothesis of the present work was that MBL influences the mannan-induced production of pro-inflammatory cytokines as well as the activation of complement, all parts of the asthmatic response. Whole blood from healthy volunteers was exposed to 100 μg/ml mannan and 100 or 500 ng/ml MBL, and human monocytes from buffy coats were exposed to 100 μg/ml mannan and 100 to 1500 ng/ml MBL for up to six hours. The MBL concentrations used were similar to those observed in mild asthma and in healthy controls. Differences between groups were tested by t-tests and two-way ANOVA with significance level $p<0.05$. In monocytes, the TNF-α and also IL-6 concentrations increased by mannan in combination with MBL (0 to 250 ng/ml), and by each of mannan and MBL alone. No further increase was seen with higher MBL concentrations. In whole blood neither MBL nor mannan induced any detectable production of TNF-α. However, mannan, but not MBL, induced the expression of CR3 (CD11b) on both granulocytes and monocytes. MBL increased the production of RANTES and PDGF in this model. These experiments suggest that MBL may interact with mannan early in an inflammatory response that involves cytokines, chemokines, growth factors and the complement system. Further experiments are needed to resolve whether healthy individuals differ from those with mild asthma in their inflammatory responses related to different levels of MBL.

PS 254 ANTIBACTERIAL AGENT TRICLOSAN INHIBITS IgE RECEPTOR-MEDIATED MAST CELL DEGRANULATION.

R. K. Palmer[1, 2], B. Burpee[2], L. Hutchinson[2], Z. Kormendy[2], E. Tupper[2], J. Pelletier[2], E. Malay[2] and J. A. Gosse[1, 2]. [1]*Graduate School of Biomedical Sciences, University of Maine, Orono, ME and* [2]*Molecular and Biomedical Sciences, University of Maine, Orono, ME.*

Triclosan (TCS) is a broad-spectrum antimicrobial that is added to hundreds of products, such as toys, cookware, and most commonly in hand soaps. TCS is an antimicrobial agent that acts through inhibition of bacterial fatty acid synthesis. In addition, an anti-inflammatory role for TCS has been detected in human skin, including for dermatitis elicited after intradermal injection of histamine (Kjcerheim et al., J Clin Peridon 1994). Symptoms of atopic dermatitis can be alleviated with TCS (Breneman et al., Cutis 2000). In the present study, we further investigate the anti-inflammatory potential and mechanism of TCS using the endpoint of mast cell degranulation. Mast cells are multifarious effector cells involved in immune response, playing an important role in the defense against parasites and serving regulatory functions in allergy, asthma, and carcinogenesis. After cross-linking of IgE receptors at the plasma membrane, a cascade of signal transduction events is initiated, culminating in degranulation, which is the release of allergic mediators, e.g., histamine, cytokines, serotonin, leukotrienes. Using a fluorescent microplate assay to measure the release of one of these mediators, β-hexosaminidase, we are able to quantify degranulation in rat mast cells (RBL-2H3). Following 1h exposure to TCS, we found a strong dose-dependent reduction in degranulation ($p<0.001$). Importantly, these same concentrations were found non-cytotoxic to RBLs in lactate dehydrogenase and clonogenic cytotoxicity assays. Confocal images of suppressed membrane ruffling support these data. We are currently investigating the molecular mechanism by which TCS inhibits mast cell degranulation. Our finding that TCS can inhibit mast cell degranulation may explain the clinical data mentioned above and suggests that TCS may be an effective treatment for allergic disease such as eczema.

PS 255 TRANSACTIVATION OF GENE EXPRESSION BY NFκB IS DEPENDENT ON THIOREDOXIN REDUCTASE ACTIVITY.

W. Watson and J. Heilman. *Department of Medicine/GI, University of Louisville, Louisville, KY.*

The redox-sensitive transcription factor NFκB mediates the expression of genes involved in inflammation and cell survival. Thioredoxin reductase-1 (TR1) and its substrate thioredoxin-1 (Trx1) act together to reduce oxidized cysteine residues within the DNA binding domain of NFκB, and to promote maximal DNA binding activity in vitro. It is not clear, however, if NFκB is regulated via this mechanism within living cells. The purpose of the present study was to determine the mechanism of NFκB modulation by TR1 in cells stimulated with the inflammatory cytokine, tumor necrosis factor-α (TNF). In both control cells and in cells depleted of TR1 activity through chemical inhibition or siRNA knock down, TNF stimulation resulted in degradation of the cytoplasmic NFκB inhibitor IκB and translocation of NFκB to the nucleus. Similarly, the DNA binding activity and redox state of NFκB were unaffected by TR1 depletion. Despite this, there was a marked inhibition of transcriptional activity of nuclear NFκB as evidenced by decreased expression of an NFκB-dependent reporter gene and an endogenous target of NFκB. These results demonstrate that TR1 activity controls the transactivation potential of NFκB, a mechanism that is distinct from the previously described Trx1-dependent regulation of DNA binding activity.

PS 256 TLR3 PROTECTS AGAINST MYOCARDITIS BY PROMOTING A TH1 RESPONSE, DECREASING VIRAL REPLICATION, PREVENTING SYSTOLIC FAILURE.

E. Abston, A. Bucek, A. Frisancho, M. Coronado, J. Shin and D. Fairweather. *Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Infectious myocarditis is a leading cause of heart failure. The development of infectious myocarditis is mediated by Toll-like receptors (TLR) in ways that are not yet understood. TLR3 is a unique pattern recognition receptor both because it re-

sponds to the endosomal dsRNA that occurs in viral infections and because it's signaling pathway is mediated entirely by TRIF – an adaptor protein implicated in the development of myocarditis that promotes an antiviral Th1 response. We hypothesize that TLR3 is uniquely positioned to play a critical role in the innate response to viral infection and that removing this receptor will result in a more severe viral infection and produce a more intense myocarditis. To test the role of TLR3 signaling in the development of myocarditis, TLR3-/- males and WT controls (N=10) were infected with heart-passaged Coxsackie Virus B3 (CVB3) to induce autoimmune myocarditis. Ten days later heart function was assessed by pressure-volume catheterization of the heart. Heart tissue was harvested to quantify inflammatory cell infiltrate, viral replication, and cytokine levels. Myocardial inflammatory cell infiltration was significantly increased by 50% in TLR3-/- mice. Viral replication increased from 10,000 PFU/g in WT to 900,000 PFU/g in TLR3-/-. Th 2 cytokines IL-4, IL-10 and TGFb1 increased, while Th 1 IFNg decreased in TLR3-/- vs WT. Heart catheterization revealed left ventricular dysfunction in TLR3-/- as characterized by: a decrease in systolic pressure from 106 mmHg in WT to 95 mmHg in TLR3-/-, a decrease in contractility (dPdT max) from 10,176 mmHg/sec in WT to 8,304 mmHg/sec in TLR3-/-, a stroke volume-dependent decrease in cardiac output from 6.2 mL/min in WT to 4.2 mL/min in TLR3-/-, and a 39% drop in stroke work in TLR3-/-. TLR3 plays a protective role in CVB3-induced myocarditis by promoting a Th 1 shift, decreasing the intensity of myocarditis, and preventing systolic failure.

PS 257 S-NITROSYLATION OF SURFACTANT PROTEIN-D OCCURS IN BLEOMYCIN-INDUCED INJURY AND IS A PRO-INFLAMMATORY MEDIATOR *VIA* ACTIVATION OF NF-κB DEPENDENT PATHWAYS.

C. Guo and A. Gow. *Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Surfactant protein-D (SP-D) is a pulmonary collectin, which plays an important role in innate immunity. Previously, we have demonstrated that SP-D is a target for nitric oxide-mediated modification. S-nitrosylation of two key cysteines in the tail domain of SP-D alters its multimeric structure. Our initial studies have shown that the S-nitrosylated form of SP-D (SNO-SP-D) associates with CD91 to activate macrophage chemotaxis. In this study, we investigated whether SNO-SP-D induces a pro-inflammatory phenotype in macrophages via activation of NF-κB. Acute lung injury model was induced by intratracheal administration of bleomycin in iNOS-/- and C57/Bl6 (WT) mice and Bronchoalveolar lavage (BAL) was collected at day 8-post injury. Bleomycin-induced lung injury was reduced in iNOS-/- mice relative to WT, as determined by a number of factors. The activity of NF-κB, as assessed by TransAM ELISA, within cells of the lung lining was reduced, relative to control, in bleomycin-injured iNOS-/- mice, as was the SNO-SP-D content. BAL from bleomycin injured mice induced NF-κB activity in Raw 264.7 cells, and this function was abrogated after removal of SNO-SP-D. SP-D over expressing BAL (OE-BAL) was harvested from Swiss-black mice over-expressing rat SP-D. S-nitrosylated OE-BAL (SNO-OE-BAL) treatment of Raw264.7 cells resulted in upregulation of IL-1β and iNOS mRNA, while OE-BAL had no effect. The SNO-OE-BAL mediated gene expression of these pro-inflammatory mediators was decreased upon SP-D depletion from the BAL. Pre-incubation of Raw264.7 with caffeic acid phenethyl ester (CAPE), a potent NF-κB inhibitor, abrogated SNO-OE-BAL mediated induction of proinflammatory genes. These findings provide a mechanism for SNO-SP-D mediated inflammatory signaling within the lung and demonstrate that this mechanism operates within an in vivo model of lung injury. Supported by ES005022 and HL086621.

PS 258 UTILIZATION OF EXTRACELLULAR GLUTATHIONE BY PHAGOCYTOSIS REDUCES TUMOR NECROSIS FACTOR α RELEASE IN MACROPHAGES.

N. S. Gould[1, 2], E. Min[1] and B. J. Day[1, 2, 3]. [1]*Medicine, National Jewish Health, Denver, Co.,* [2]*Pharmaceutical Sciences, University of Colorado AMC, Aurora, CO and* [3]*Immunology, University of Colorado AMC, Aurora, CO.*

The epithelial lining fluid (ELF) of the lung is an important protective barrier against inhaled toxic chemicals or pathogens. The ELF maintains high levels of glutathione (GSH) which can protect the lung by detoxifying oxidants. Additionally, GSH has been shown to have a role in modulating inflammatory responses in epithelial cells. One of the major cell types found in the ELF are alveolar macrophages, which when stimulated can be a major source of oxidants and proinflammatory cytokines. There is a positive correlation between ELF GSH and alveolar macrophage intracellular GSH but it is unclear how GSH in the extracellular environment is able to be utilized by macrophages and whether it attenuates cytokine release. When macrophages are grown in media supplemented with 500 μM GSH, there is a 2-3 times increase in intracellular GSH. This increase in intracellular GSH corresponds to a significant reduction in NF-κB nuclear translocation and tumor necrosis factor α (TNFα) release when stimulated with LPS. Furthermore, treatment with acivicin or buthionine-sulfoximine, inhibitors of GSH breakdown and synthesis, did not block GSH uptake. In contrast, cytochalasin D, an inhibitor of phagocytosis, blocked the increase in intracellular GSH and blocked the attenuation of TNFα release. Furthermore, in vivo, the activation of alveolar macrophages is increased with cigarette smoke in aged mice with decreased GSH levels leading to an increase in TNFα levels. These data suggest that macrophages are able to utilize extracellular GSH through phagocytosis which can then modulate inflammatory signaling in response to proinflammatory stimuli. Supported in part by NIH grant HL084469 (BJD).

PS 259 ACROLEIN INHIBITS THE CLEAVAGE OF ADHESION MOLECULES IN CYTOKINE-STIMULATED ENDOTHELIAL CELLS.

S. D. Sithu, A. Bhatnagar, S. Srivastava and S. E. D'Souza. *School of Medicine, University of Louisville, Louisville, KY.*

Acrolein is an α,β-unsaturated aldehyde abundant in automobile exhaust, tobacco smoke and industrial waste. It is also present in high quantities in several beverages and food. Due to its α,β-unsaturation, acrolein is a strong electrophile and readily reacts with cellular nucleophiles such as the -SH residues of reduced glutathione (GSH). Depletion of GSH perturbs the redox state of the cells and has been implicated in vascular dysfunctions. We observed that incubation of endothelial cells (EC) with acrolein (0-10 μM; 1h) in serum-free medium followed by stimulation with TNF-α (15ng/ml) or IL-1 after 24h, increased the cell surface expression of ICAM-1 and VCAM-1 by 1.5-2 fold. However, the soluble ICAM-1 (sICAM-1) and sVCAM-1 levels in the media were significantly lowered (20-90% for 2.5-10μM acroline). The adhesion of monocytic cells to acrolein exposed EC as well as the transmigration of the monocytic cells across acrolein exposed EC were significantly increased as compared with untreated EC. GSH levels in acrolein-treated EC was depleted by 50% at 1h and returned to normal levels by 6h. Similar to acrolein, incubation of EC with the 9C α,β-unsaturated aldehyde 4, hydroxy trans-2-nonenal (HNE), increased the cell surface expression of ICAM-1, increased monocyte adhesion and transmigration, but decreased the cleavage of ICAM-1. Moreover, tertiary butyl hydroperoxide (t-BHP, 0-100 μM), a well known oxidant, significantly depleted GSH levels in EC and decreased the concentration of sICAM-1 in the cell culture medium. sICAM-1 and sVCAM-1 levels in the plasma of acrolein (5mg / kg for 24h by gavage) exposed mice challenged with LPS (1mg/kg) were decreased by 15-20% as compared with the controls, gavage-fed with water. The lower levels of sICAM-1 and sVCAM-1 seen upon exposure to α,β-unsaturated aldehydes acrolein and HNE, and the non aldehydic oxidant t-BHP, in cytokine stimulated EC suggest a relationship between GSH depletion and cytokine signaling that regulate the processing of adhesion molecules affecting EC function.

PS 260 INDUCTION OF PROINFLAMMATORY MEDIATORS FACILITATES PCB-MEDIATED ENHANCEMENT OF BRAIN METASTASIS FORMATION.

E. Bodone Sipos, L. Chen, I. Andras, B. Zhang, J. Wrobel, M. Park, J. Choi, S. Eum and M. Toborek. *Molecular Neuroscience and Vascular Laboratory, Department of Neurosurgery, University of Kentucky, Lexington, KY.*

Polychlorinated biphenyls (PCBs) are environmental toxicants that can cause vascular inflammation. Our research has focus on a general hypothesis that exposure to PCBs can promote the development of brain metastases by altering the blood brain barrier (BBB) integrity. The crucial event in tumor cell extravasation is the arrest of the blood-borne tumor cells in the capillary bed. This process is followed by adhesion of tumor cells to the vascular endothelium and their transcapillary migration. In the present study, we evaluated the hypothesis that PCB-induced inflammatory processes at the BBB level can facilitate the formation of brain metastases. PCB118 was administered orally to mice at the dose of 150 μmol/kg. Forty eight hours later, mice were injected with Lewis lung carcinoma cells tagged with luciferase into the carotid artery. Brain tumors were detected by an in vivo imaging system. In addition, expression of VCAM-1, ICAM-1, and MCP-1 was assessed by real-time PCR (rtPCR) and immunofluorescence microscopy in isolated brain microvessels 6 h or 27 days after tumor cell injection. In vivo imaging demonstrated a potent development of brain metastases during the one month monitoring period, with a marked increase in metastasis formation in mice treated with PCB118 as compared to vehicle treated animals. Our rtPCR and immunostaining results indicated that injection of tumor cells induced the expression of VCAM-1, ICAM-1, and MCP-1 and that these effects were potentiated by exposure to PCB118. The results of the present study suggest that exposure to PCBs, such as PCB118, may induce the migration of tumor cells across the BBB via a process facilitated by proinflammatory mediators and leading to potentiation of brain metastasis formation. Supported by P42 ES 07380.

PS 261 NITRIC OXIDE ENHANCES BACTERIAL CLEARANCE IN HYPEROXIA.

A. Gore. *St. John's University, Queens, NY.*

A significant number of patients on ventilator develop ventilator-associated pneumonia (VAP) and inflammatory lung injury. Despite the advancement of antimicrobial therapy, VAP results in significant morbidity and mortality in these patients. Previously, studies including ours have demonstrated exposure to prolonged hyperoxia results in macrophage dysfunction. In this study, we observed that prolonged hyperoxia suppressed levels of airway NO. We tested whether administration of exogenous NO enhances the host's ability to clear the bacteria. C57BL/6 mice were randomized to receive either intranasal DETA-NONOate (D-NO), a NO donor, or control solution, exposed to ≥99.0% O2, and then inoculated with intranasal instillation of PA. Treatment with D-NO improved the survival, decreased bacterial load in the lung and mitigated hyperoxia/PA-induced lung injury in a dose-dependent manner. Moderate concentrations of D-NO significantly rescued hyperoxia-suppressed macrophage functions in RAW 264.7 cells exposed to 95% O2. However, macrophages exposed to D-NO at concentrations of 150 μM or higher underwent cell death. Our previous work demonstrates that hyperoxia induces HMGB1 release, which contributes to hyperoxia-compromised macrophage dysfunction and is partly mediated by NF-κB. D-NO inhibited hyperoxia-induced HMGB1release and NF-κB activation in RAW 264.7 cells. These results indicate that exogenous NO might provide a novel therapeutic approach in preventing patients on ventilator to develop VAP.

PS 262 ENDOTHELIAL ACTIVATION BY 4, HYDROXY TRANS-2-NONENAL: ROLE OF ENDOPLASMIC RETICULUM STRESS.

E. N. Vladykovskaya, S. D. Sithu, P. Haberzettl, N. S. Wickramasinghe, O. A. Barski, S. E. D'Souza, A. Bhatnagar and S. Srivastava. *School of Medicine, University of Louisville, Louisville, KY.*

Endothelial activation is an early event in atherogenesis. Although the mechanisms leading to endothelial activation in atherogenesis remain poorly understood, extensive evidence suggests that activation of the endothelium in lesion-prone sites is due to the sub-endothelial activation of oxidized LDL. We observed that 4-hydroxy-trans-2-nonenal (HNE; 25 μM), one of the most abundant and reactive aldehyde, generated from the oxidation of LDL and membrane lipids increased the cell surface expression of ICAM-1 on endothelial cells (EC) by 1.3-fold, augmented monocyte adhesion to EC (2-fold) and their transmigration through EC (2-fold), and increased the expression of pro-inflammatory cytokines (TNF-α, IL-6 and IL-8) by 4-12-fold. HNE also modified several proteins in EC. Confocal imaging of HNE-treated EC showed that the modified proteins were associated with the ER. Exposure to HNE led to the splicing of the bZIP transcription factor, XBP-1, a characteristic feature of the alarm phase of ER stress, and an increase in the phosphorylation of eIF2α, which regulates the expression of multiple ER-stress dependent transcription factors. These transcription factors, ATF3 and ATF4, were also strongly (3-5 fold) induced by HNE. Treatment with HNE was associated with a 2-5 fold increase in the expression of ER chaperones, GRP78 and HERP, indicating that HNE triggers the adaptive phase of the response, which increases the protein folding capacity of the ER. Adenoviral transfection with ATF6 increased the expression of molecular chaperones Grp78, Grp94 and PDI and attenuated HNE-induced monocyte adhesion to EC and transcription of IL-8. Similarly, pre-treatment with the chemical chaperone, phenylbutyric acid (PBA), to facilitate protein folding, attenuated HNE- induced monocyte adhesion and transcription of IL-8. These data suggest that HNE- induced ER stress could be a critical regulator of endothelial function.

PS 263 LOVASTATIN ATTENUATES HYPEROXIA-INDUCED HMGB1 RELEASE AND RESCUES MACROPHAGE FUNCTION *VIA* INACTIVATION OF NF-κB P65 PATHWAY.

R. Malla[1] and L. Mantell[1, 2]. [1]*St. John's University, Queens, NY and* [2]*The Feinstein Institute for Medical Research, North Shore-LIJ Health System, Manhasset, NY.*

Mechanical ventilation with supraphysiological concentrations of oxygen (hyperoxia) is routinely used in treating both newborns and adults with respiratory insufficiency. However, exposure to prolonged hyperoxia is associated with increased susceptibility to microbial infection. Our previous studies also demonstrate that HMGB1 (a pro-inflammatory cytokine) from macrophages exposed to hyperoxia results in decreased ability to clear micro-organisms in mouse model of Pseudomonas aeruginosa. Statins, HMGcoA reductase inhibitors, have been shown to reduce elevated inflammatory cytokines like IL-6, IL-8 and TNF-alpha in hypercholesterolemic patients. The purpose of this study was to investigate whether Lovastatin, a Statin analogue, affects hyperoxia-induced release of HMGB1 and function in cultured RAW 264.7 macrophages. We tested the hyperoxia-suppressed ability of macrophages to phagocytose microbes by pretreatment with a series of concentrations of Lovastatin and then expose to 95%O2 for 24 hr. Phagocytosis and HMGB1 release was determined by immunofluorescence and Western Blot analysis respectively. The release of HMGB1 under hyperoxic conditions was inhibited by Lovastatin in a dose-dependent manner. Corresponding to the decreased release of HMGB1, Lovastatin significantly enhanced macrophage's ability to phagocytose under hyperoxic conditions. To further investigate whether attenuating effects of Lovastatin was NF-κB mediated, we studied NF-κB translocation on hyperoxia-suppressed macrophages via immunofluorescence . These results indicate that Lovastatin can effectively reduce both hyperoxia-induced inflammation mediated by HMGB1 and rescue hyperoxia-suppressed macrophage function. Also, it implicates that HMGB1 release is atleast partly mediated by NF-κB under hyperoxia. Therefore, lovastatin might be clinically employed to treat patients on ventilation with inflammatory lung injury and ventillator-associated pneumonia.

PS 264 SELECTIVE ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST GTS-21 ENHANCES HYPEROXIC MACROPHAGE FUNCTION.

R. A. Sitapara. *Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY.* Sponsor: L. Mantell.

Macrophages are the first line of defense against invading pathogens. However, exposure to prolonged hyperoxia compromises host ability to phagocytose bacteria. Our previous studies show that exposure to prolonged hyperoxia leads to HMGB1 release and HMGB1 suppresses macrophage's ability to phagocytose. The vagus nerve of autonomous nervous system regulates the levels of proinflammatory cytokines, thus modulating inflammation-mediated damage. Dr. Kevin Tracey's lab has shown that 3(2,4 dimethoxybenzylidene) anabaseine (GTS-21), an α7 nicotine acetylcholine receptor agonist inhibits endotoxin-induced HMGB1 release from RAW 264.7 cells (a macrophage like cell line). The aim of this study was to examine whether GTS-21 could inhibit hyperoxia-induced HMGB1 release and enhance macrophage function under hyperoxic condition. RAW 264.7 cells were exposed to different concentrations of GTS-21 prior to exposure to 95% O_2.Phagocytosis assay was performed to determine macrophage function. HMGB1 release was assessed by Western blotting and immunofluroscence analyses. Nuclear factor kappa B (NFκB)has been shown to modulate endotoxin-induced HMGB1 release. Activation of NFκB was assessed by its translocation from cytoplasm to nuclei by immunofluroscence assay. We show here that GTS-21 dose dependently inhibited HMGB1 release and NFκB translocation, and enhanced the ability of macrophage to phagocytose. These results indicate that GTS-21 is effective in inhibiting hyperoxia-induced HMGB1 release and enhancing hyperoxic macrophage function.

PS 265 METALLOTHIONEIN AND ACUTE CADMIUM-INDUCED DNA OXIDATIVE DAMAGE.

W. Qu[1], L. Cheng[1], Y. Sun[1], R. P. Mason[2] and M. Waalkes[1]. [1]*NTP Laboratories, NTP, NIEHS, Research Triangle Park, NC and* [2]*Laboratory of Toxicology and Pharmacology, NIEHS, Research Triangle Park, NC.*

Metallothionein (MT) plays an important role in detoxication of heavy metals such as cadmium (Cd), a human carcinogen. The mechanisms by which MT may impact Cd carcinogenesis remain poorly understood. Thus, we studied the role of MT in acute Cd-induced oxidative DNA damage (ODD). MT-I/II double knockout (MT-null) and parental wild-type (WT) cell lines were treated with Cd (as CdCl2) for 24 h and cytotoxicity was measured. Cd was much less cytotoxic in WT cells (LC50 = 16.5 ± 2.1 μM, mean ± SEM) than MT-null cells (LC50 = 6.6 ± 1.6 μM). Cd-induced ODD was measured using the immuno-spin trapping method which directly traps DNA radicals by using a spin-trap agent to form a stable DNA nitrone adduct in situ from the radicals. Cd treatment (1 or 5 μM; 24 h) induced much less ODD in WT cells (114% and 137% of control, respectively) compared to MT-null cells (298% and 438%). Cd caused concentration-related increases in MT expression in WT cells (transcript and protein). In contrast, the basal levels of MT were very low and were not increased by Cd exposure in MT-null cells. Cd treatment increased expression of two important transport genes, Mrp1 and Mrp2, in WT cells but not MT-null cells. Cd caused concentration-related increases in oxidant defense genes, such as HO-1 and GSTa2, in both WT and MT-null cells. However, the increased expression was much higher in MT-null cells, where, for example, both HO-1 and GSTa2 maximal transcript levels after Cd treatment were 3-fold higher than WT cells. Treatment with the direct oxidant, hydrogen peroxide, at 0.25 and 0.5 mM (24 h) also caused a concentration-dependent increase of

ODD in both WT (maximum 177% of control) and MT-null cells (maximum 276% of control) but ODD levels were markedly lower in WT cells, in a fashion similar to that for Cd treatment. Thus, MT protects against Cd-induced and oxidant-induced ODD possibly by multiple mechanisms, including sequestration of the metal ion and of oxidant radicals. MT-null cells may adapt to Cd by turning on the oxidant response system.

PS 266 SEXUAL DIMORPHISM OF CADMIUM-INDUCED TOXICITY IN RATS: INVOLVEMENT OF PROGESTERONE.

H. Shimada[1], T. Hashiguchi[1], A. Yasutake[2], M. P. Waalkes[3] and Y. Imamura[4]. *[1]Kumamoto University, Kumamoto, Japan, [2]National Institute for Minamata Disease, Kumamoto, Japan, [3]NIEHS, Research Triangle Park, NC and [4]Nihon Pharmaceutical University, Saitama, Japan.*

The toxic effect of cadmium (Cd) varies with sex in experimental animals. Our recent studies have demonstrated that the pretreatment with female sex hormone progesterone markedly enhances the susceptibility to Cd toxicity in Fischer 344 (F344) rats, suggesting a role for progesterone in the sexual dimorphism of Cd toxicity. In the present study, we further attempted to elucidate the mechanism for sex difference of Cd-induced toxicity in F344 rats. A single administration of Cd (5.0 mg/kg, sc) was lethal in 0/10 (0 %) female compared to 4/10 (40 %) male rats. Using lower dose of Cd (3.0 mg/kg), circulating alanine aminotransferase (ALT) activity, indicative of hepatotoxicity, was much higher in the Cd treated females than in the males. However, no difference in the hepatic Cd accumulation, metallothionein (MT) or glutathione (GSH) levels were observed between male and female rats. When Cd (5.0 mg/kg) was administered to young rats at 5 weeks of age, sex-related difference was hardly recognized for the Cd-induced lethality. Furthermore, although ovariectomy decreased the Cd-induced lethal toxicity, the decreased effect was restored by the pretreatment with progesterone (40 mg/kg, sc, 7 consecutive days) to ovariectomized rats. These results provide further evidence that progesterone is involved in the sexual dimorphism of Cd toxicity in rats.

PS 267 CADMIUM ACCUMULATES WITHIN PANCREATIC ISLETS RESULTING IN IMPAIRED GLUCOSE STIMULATED INSULIN SECRETION IN AN ANIMAL MODEL OF SUBCHRONIC EXPOSURE.

J. Edwards, R. Fitzgerald and A. Olsen. *Department of Pharmacology, Midwestern University, Downers Grove, IL.*

There is increasing interest to understand how environmental contaminants can contribute to the onset of diabetes. Recent epidemiological studies show that exposure to the metal cadmium (Cd), is associated with diabetes and reduced serum insulin. The goal of this study was to examine the effects of Cd on insulin secretion using a well established animal model of sub chronic Cd exposure. Male Sprague Dawley rats were injected (s.c.) daily with either saline (control) or Cd (0.6 mg Cd/kg/day, 5 days per week). After weeks 9 and 12 of treatment tissue samples were analyzed for Cd content, serum insulin levels, and pancreatic islets were isolated. Previous experimental studies report Cd accumulation in the whole pancreas (islets + acinar cells) to be about 20% of that found in the renal cortex. In contrast, the current study shows that islet cells alone contain levels of Cd similar to that found in the renal cortex. Specifically, the islets contained 69% of the total amount of Cd in renal cortex at week 9 and 81% at week 12. This accumulation of Cd within the islets had deleterious effects on insulin release. The mean fasting serum insulin value was 33% lower in the Cd treatment group at week 9. In another experiment, islets were isolated and incubated in various glucose solutions. The amount of insulin release from islets isolated from control animals was approximately one fold higher when incubated in high (3.0mg/ml) glucose solutions as compared to low (0.5mg/ml) glucose, indicating the islets were normal and healthy. Conversely, islets isolated from Cd treated animals resulted in a 16% to 26% decrease in insulin release when islets were incubated in high glucose solutions as compared to low. This study indicates that Cd accumulates to a greater degree in pancreatic islets than previously thought. Furthermore, this Cd accumulation may result in islet dysfunction as evidenced by lower fasting serum insulin levels and blunted physiological responses such as glucose stimulated insulin release.

PS 268 CADMIUM ALTERS MICRORNA EXPRESSION IN HUMAN A549 ALVEOLAR EPITHELIAL CELLS.

M. J. Fay, P. Rodriguez, L. A. Alt and W. C. Prozialeck. *Pharmacology, Midwestern University, Downers Grove, IL.*

Acute respiratory exposure to Cd results in pulmonary edema and hemorrhaging, which is later followed by inflammation, scarring and fibrosis. Long-term respiratory exposure has been associated with development of chronic obstructive pulmonary disease, and possibly, some forms of lung cancer. While the general toxic effects of Cd in the lung have been well-characterized, the specific molecular mechanisms underlying these effects have yet to be fully elucidated. MicroRNAs are small noncoding RNAs that decrease gene expression at the post-transcriptional level. Alterations in the microRNA expression profile have been implicated in both cellular/tissue toxicity and cancer development. In this study we investigated the effects of Cd exposure on microRNA expression in the human alveolar epithelial A549 cell line. This cell line, which exhibits many characteristics of type-II pneumocytes, has been extensively used as an in vitro model for Cd-induced pulmonary injury. Subconfluent (70-80%) cells were exposed to CdCl2 (10 microM) for 24 hrs in serum-free medium. Cytotoxicity was evaluated using trypan blue and hemacytometer cell counting, and WST-1 assays. MicroRNA array analysis showed that acute exposure to a relatively non-cytotoxic concentration of Cd caused a statistically significant (t-test, $P< 0.05$) alteration in the expression of 7 human MicroRNAs (miR-1308, miR-3127, miR-23b, miR-23a, miR-92b, miR-222, and miR-1915). Northern blot analysis for miR-1308 confirmed the array results and indicated increased expression of miR-1308 with Cd-treatment. These finding suggest that alterations in the microRNA expression profile may play a role in the pathophysiology of Cd-induced lung injury.

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PS 269 SUPPRESSION OF IRON TRANSPORT SYSTEM IN DUODENUM BY CADMIUM.

M. Satoh, H. Banno and Y. Fujiwara. *Laboratory of Pharmaceutical Health Sciences, School of Pharmacy, Aichi Gakuin University, Nagoya, Japan.*

It is known that cadmium (Cd) causes iron deficiency anemia, but the mechanism is not clear. In this study, to elucidate the mechanism of Cd-induced iron deficiency anemia, we examined the effect of Cd on the expression of iron transport-related genes in the duodenum of mice. Eight-week-old female C57BL/6J mice were given a single oral administration of Cd at a dose of 50 mg/kg and were sacrificed at 3 and 24 h after the Cd administration. The mRNA levels of duodenal cytochrome b (Dcytb), divalent metal transporter 1 (DMT1), ferroportin 1 (FPN1) and hephaestin (HEP), which are iron transport-related genes, in the duodenum were determined using real-time RT-PCR analysis. Serum iron concentration was measured using Fe C-test wako kit (Wako Pure Chemicals, Osaka, Japan). The mRNA levels of Dcytb, DMT1, FPN1 and HEP in the duodenum were remarkably decreased at 3 h after the Cd administration. On the other hand, serum iron concentration was significantly reduced to 24 h after the Cd administration. The present study clearly found that Cd inhibits the expression of iron transport-related genes in the duodenum of mice. These results suggest that Cd may inhibit iron absorption through suppression of iron transport system in the duodenum.

PS 270 COMBINED EFFECT OF ABDOMINAL SURGERY AND CHRONIC CADMIUM ADMINISTRATION ON HEPATIC RIBONUCLEASE ACTIVITY, METALLOTHIONEIN, AND ALPHA-2-MACROGLOBULIN CONCENTRATION.

E. Brambila, M. R. Vieyra-Lobato, B. A. Leon-Chavez and P. Aguilar-Alonso. *Clinical Chemistry, University of Puebla, Puebla, Puebla, Mexico.*

Ribonucleases (RNases) are a group of enzymes that hydrolyze different classes of RNA and its activity regulates indirectly intracellular protein synthesis. Earlier we described that hepatic acid RNase activity is decreased after an experimental surgery, which can be an important event to favor acute phase protein synthesis, including metallothionein (MT). In addition, several studies in vitro have shown that cadmium (Cd) inhibit RNase activity by an interaction of Cd with amino acids near the active site. In this work we studied the combined effect of inflammation and chronic cadmium administration in rats on hepatic RNase activity, MT and alpha-2-macroglobulin synthesis. Rats (groups of 5) were administered i.p. with 0.25 and 0.5 mg of Cd/Kg by 15, 30, 60 and 90 days, and then each rat received and abdominal 3 cm incision. Controls were administered with sterile water or 0.5 mg of Cd/Kg, but no surgical treatment. Hepatic RNase activity, Cd, MT and alpha-2-macroglobulin were measured. Results obtained shown a time-concentration liver accumulation of Cd, same pattern was observed with MT liver concentration, however, liver RNase activity decreased significantly in relation with the accumulated Cd. These results suggest that combination of inflammation and chronic exposure of Cd inhibit RNase activity to increase protein synthesis, which is confirmed with the observed increase of alpha-2-macroglobulin in our animal model.

PS 271 CIRCADIAN VARIATION OF CADMIUM-INDUCED MORTALITY IN MICE.

N. Miura. *Japan National Institute of Occupational Safety and Health (JNIOSH), Kawasaki, Japan.* Sponsor: M. Satoh.

Mammalian circadian rhythms are regulated by subtle feedback loop system of clock genes, the major molecular time-keeping apparatuses. The circadian system drives daily rhythmic changes in multiple physiological and biochemical processes such as sleep, hormonal secretion, metabolism, cell cycle, DNA repair, apoptosis, etc. It is recognized that several chemical compounds including anticancer drugs (cisplatin and 5-FU) show circadian variations of toxicity due to circadian rhythm of metabolic enzymes activity. In case of metal compounds, this circadian variation is poorly investigated. This study reports the circadian susceptibility to cadmium in mice. ICR mice (n=10, male, 6 w), kept in cages on 8:00-20:00 L/D cycle, were administrated CdCl2 (7.2 mg/kg body weight; half lethal dose injected at 12:00 determined by preexamination) intraperitoneally at different hours in the day (every four hours: 12:00, 16:00, 20:00, 0:00, 4:00 and 8:00). The mortality was determined during 10 consecutive days. Mice were most sensitive to CdCl2 administrated at 16:00 and 20:00, while they were least sensitive at 0:00 and 4:00, late-night to early morning. The analysis of the relation between the oscillation in cadmium sensitivity and the concentrations of metallothionein or glutathione in liver and serum is in progress. A circadian susceptibility to metals such as cadmium clearly appears, thus it is necessary to clarify the mechanism with contemplating for the clock genes.

PS 272 TOXIC EFFECTS OF CADMIUM ON HUMAN PERIPHERAL LUNG CELLS.

R. Person[1], E. Tokar[2] and M. Waalkes[2]. *[1]NCI at NIEHS, Research Triangle Park, NC and [2]National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Cadmium is a known human carcinogen and evidence indicates it causes lung tumors in humans and rodents. Cellular absorption of cadmium often activates the synthesis of metallothioneins (MTs), which may increase cadmium's overall bioavailability. Currently, we are attempting to develop an in vitro model of cadmium-induced lung carcinogenesis by chronically exposing the human peripheral lung cell line, HPL-1D, to low level cadmium in an attempt to stimulate acquired malignant phenotype. Here, we studied MT production to assess altered cadmium biokinetics during the transformation process. Initial studies showed that the lethal concentration in 50% of cells (LC50) for cadmium in HPL-1D cells was 13 μM. HPL-1D cells were then chronically exposed to 5 μM cadmium and monitored for signs of acquired malignant phenotype. RT-PCR was used to measure transcript levels of various genes of interest and matrix metalloproteinases-2 (MMP-2) transcript was used as a screen for cancer phenotype. Results indicated a 2-fold increase over control in MMP-2 transcript levels even at 2 weeks of exposure to cadmium. Since MT likely enhances cadmium bioaccumulation, the major human MT isoforms, MT-1A and MT-2A, were assessed. Data indicated a 475% and 2585% increase in MT-1A and MT-2A transcripts, respectively, over control after 2 weeks of cadmium exposure. Moreover, by 4 weeks of chronic exposure these same transcript levels were still highly elevated, with MT-1A at 591% and MT-2A at 378% of control. This suggests MT binds a large surplus of intracellular cadmium and facilitates cellular accumulation of the metal. Transcript for the solute carrier gene SLC30A1, also known as zinc transporter-1 (ZNT-1), was increased to 330% above control at 2 weeks and 372% at 4 weeks. These data suggest that it is possible for ZNT-1 to transport cadmium into the cell and facilitate bioaccumulation. In conclusion, these data suggest that exposure of human lung cells to cadmium may have effects that rapidly enhance cadmium bioaccumulation and reflect early acquisition of malignant phenotype.

PS 273 GENETIC TOXICITY OF HEXAVALENT CHROMIUM IN THE GASTROINTESTINAL TRACT FOLLOWING ORAL EXPOSURE.

T. J. O'Brien[1], H. Ding[1], S. R. Patierno[1], R. D. May[2] and C. D. Hébert[2]. *[1]Pharmacology and Physiology, George Washington University, Washington, DC and [2]Southern Research Institute, Birmingham, AL.*

Occupational exposure to hexavalent chromium [Cr(VI))]-containing compounds has been shown to cause lung cancer. Recently Cr(VI) has been shown to induce tumors of the gastrointestinal tract (GI) of rodents following drinking water exposure at concentrations exceeding 60 mg/L sodium dichromate dihydrate (SDD). Both genotoxicity and mutagenicity are expected to play a role in carcinogenicity of Cr(VI)-induced GI tumors, but the role that these endpoints play in the mode of action (MOA) of Cr(VI) is uncertain. To investigate the genotoxicity and mutagenicity of Cr(VI) in the GI tract of rodents, SDD at 0, 0.3, 4, 14, 60, 170, 520 mg/L was administered ad libitum for 7 or 90 days. Samples were analyzed for 8-Oxo-2'-deoxyguanosine (8-OHdG) at 90 days (10 animals/group), and Cr-DNA binding at 7 and 90 days (5 animals/group) to assess genotoxicity. For both 8-OHdG and Cr-DNA binding, DNA was isolated from scrapings of the oral, duodenal and jejunal tissues. 8-OHdG analysis was performed using an ELISA, and Cr-DNA binding was measured by ICP/MS. 8-OHdG levels were 2-3 times higher in the duodenal epithelium versus oral mucosa; however, a concentration-dependent increase in oxidized DNA bases was not observed. Concentration-dependent increases in Cr-DNA binding were observed in jejunal and duodenal epithelium at 7 days. Data collected to date indicate that Cr(VI) is genotoxic in intestinal epithelium of mice through direct interactions between Cr and DNA rather than through oxidation of DNA bases. Data for Cr-DNA binding following 90 days of exposure in mice, as well as complementary observations in rat tissues, and results of mutation analysis will be discussed relative to the role of genotoxicity in the MOA for Cr(VI) carcinogenicity.

PS 274 IDENTIFICATION OF CADMIUM-RESPONSIVE TRANSCRIPTIONAL REGULATORS OF THE *C. ELEGANS* GENE, CDR-1.

L. E. Fuhrman and J. H. Freedman. *NIEHS, Durham, NC.*

Cadmium is a toxic transition metal that can produce a variety of human pathologies. Several detoxification genes are up-regulated in C. elegans in response to cadmium, including cdr-1. Although currently there are no known mammalian homologs of cdr-1, it is likely that components of metallo-regulatory pathways controlling cdr-1 transcription are conserved. Therefore, defining the mechanism of cdr-1 regulation is crucial in understanding mechanisms of cadmium-responsive transcription in higher organisms. Forward and reverse genetic screens were designed to identify pathways regulating cadmium-responsive cdr-1 transcription. For these screens, an integrated transgenic strain of C. elegans containing GFP under the control of the 5'-regulatory region of cdr-1, pcdr-1::GFP, was used. In the absence of cadmium, pcdr-1::GFP expression was not observed in adult nematodes. However, after exposure to 10μM CdCl2 for 24 h, pcdr-1::GFP expression occurred throughout the intestine. In a reverse genetic study, the effect of loss of genes involved in regulating cadmium-responsive cdr-1 transcription was determined. Many genes involved in stress response, such as hsf-1, sir-2.1, atf-7, and MAP kinases did not affect cdr-1 expression. The daf-2/daf-16 pathway is involved in the regulation of aging and various stress responses. Knocking down the daf-16 kinase complex, akt-1/akt-2/sgk-1 resulted in significantly lower expression of pcdr-1::GFP, however, loss of other components of this pathway, such as daf-2, daf-16, or pdk-1 did not alter cdr-1 transcription. This indicates that this complex is involved in regulating cdr-1 in a daf-2/daf-16-independent manner. To identify additional genes involved in cdr-1 regulation, a forward genetic EMS screen was conducted. A total of 28 mutants that expressed reduced levels of pcdr-1::GFP and 23 mutants that expressed increased levels of pcdr-1::GFP were isolated. We are currently utilizing both SNP mapping and NextGen sequencing to identify the genes associated with the mutant phenotype in these strains.

PS 275 CHARACTERIZATION OF THE MOLECULAR MECHANISM OF CADMIUM AND NICKEL-INDUCED TOXICITY.

B. Longwell and M. Reynolds. *Biology, Washington College, Chestertown, MD.*

Cadmium and nickel are toxic metals which cause organ system damage as well as carcinogenesis in those exposed. These metals are unavoidable in modern society as they are both key components in various industrial applications. The mechanisms of toxicity for these metals include the formation of reactive oxygen species (ROS) and associated oxidative damage to genetic material, as well as the inhibition of DNA repair mechanisms. Most studies have investigated the effects of single metal exposure however, in order to mirror actually environmental exposure, this study examines the cytotoxic, genotoxic, and mutagenic effects of dual exposure to cadmium and nickel in H460 human lung epithelial cells. The cytotoxic effects of dual exposure were examined using colony formation assays and trypan blue exclusion assays. The mutagenic effects of cadmium and nickel were monitored by observing the presence of micronuclei in exposed cells. Results indicated that dual exposure was cytotoxic and mutagenic in a dose-dependent manner; more significantly than single exposure to cadmium. Analysis of dual exposed cells indicated that apoptosis was mediated via caspase 3, 7 and 8 activation and subsequent PARP cleavage. Furthermore, the upregulation of γ-H2AX, a marker of double strand break production, was observed supporting the role of ROS as a mechanism of toxicity.

Lastly, using p53siRNA cells, p53 was found to have a significant role in dual exposure apoptosis. Overall, this study confirmed that exposure to cadmium and nickel is more toxic than exposure to cadmium alone and increases our knowledge of the mechanisms of cadmium/nickel induced carcinogenesis.

PS 276 CADMIUM EXPOSURE ALTERS THE EXPRESSION OF KEY REGULATORY GENES IN OSTEOBLASTIC CELLS *VIA* ACTIVATION OF THE ERK SIGNALING PATHWAY.

M. A. Simmonds, C. M. Christensen, D. L. Filer, D. E. Haggard and S. J. Heggland. *Biology, The College of Idaho, Caldwell, ID.*

Reports of cadmium in electronic waste and jewelry have increased public awareness about exposure to this toxic heavy metal. Human exposure to cadmium is associated with the development of bone diseases, such as osteoporosis. We previously reported that cadmium exposure leads to a decrease in RUNX2, a key transcriptional factor essential for the maintenance of osteoblasts. The goals of this study were to examine the effect of cadmium on other key osteoblast genes, including alkaline phosphatase (ALP), sclerostin (SOST), and osteopontin and to elucidate the signaling pathway(s) involved. We hypothesize that cadmium exposure disrupts osteoblast function by activating the extracellular signal regulated kinase (ERK) signaling pathway leading to altered gene expression. Cells were cultured in the presence or absence of 10 μM CdCl2 for 1-30 hours and protein and mRNA expression were assessed by Western blot analysis and qPCR, respectively. We detected significant ERK activation in response to 10 μM CdCl2 at early and later time points in tumor-derived Saos-2 cells (pERK/ ERK relative density control = 0.21 ±0.03 compared to CdCl2 = 0.48 ±0.05 at 18 hour exposure). Cells exposed to CdCl2 also exhibited a significant decrease in ALP and osteopontin mRNA compared to untreated cells. In contrast, SOST mRNA significantly increased in response to CdCl2. In order to assess whether the ERK signaling pathway is linked to altered gene expression, we pretreated cells with the ERK inhibitor 5μM PD98059 followed by 10 μM CdCl2 for 18 hours. Preliminary results indicate that pretreatment with the ERK inhibitor has a recovery effect on cadmium-induced altered ALP and SOST mRNA expression. Current studies include assessing cadmium's impact on cells induced to form a mineralized matrix. Collectively, this research provides further evidence that cadmium directly impacts bone and thus is a risk factor for osteoporosis. Research funded by NIH-INBRE P20RR016454 and NIH R15ES015866 grants.

PS 277 EFFECTS OF CIGARETTE SMOKE CADMIUM AND SMOKING CESSATION ON URINARY CALCIUM EXCRETION IN POSTMENOPAUSAL WOMEN.

M. H. Bhattacharyya[1, 2] and S. Bozorgnia[2]. [1]*Medicine, Medical College of Georgia, Augusta, GA and* [2]*Orthopedic Surgery, Medical College of Georgia, Augusta, GA.*

Low-level cadmium (Cd) exposure in humans is correlated with decreased bone density and increased susceptibility to fractures. In addition, cigarettes are a major source of Cd exposure. This study investigates if the Cd component of cigarettes is associated with increased calcium excretion from the kidneys of persons who smoke. Eighteen postmenopausal women who smoke cigarettes and 11 non-smokers were studied. From each participant, 2 blood and 11 urine samples were collected over 7 wks: the first 3 wks during which smokers smoked at their customary rate; the next 4 days when all smokers ceased smoking; the next 4 wks when smokers, to different degrees, returned to previous smoking status. Blood was collected at the beginning and end of the 7 wks. A first-of-morning (FOM) urine specimen was collected 1- to 2-times per wk during the 7 wks. Smoking status was documented by daily diary and verified with CO breath test. Analyses included CdB, urine Cd concentration (CdU), and urine calcium concentration (CaU). Results demonstrate that mean values of CdB and CdU were significantly higher in smokers than non-smokers (3.5- and 2.6-fold, respectively). Prior to smoking cessation, Cd exposure did not increase mean levels of FOM CaU, with 57±7 (9), 60±14 (8), and 54±10 (11) mg Ca/sample for higher-Cd smokers (CdB, 1.0-2.7 ng Cd/ml), lower-Cd smokers (CdB, 0.5-0.9 ng Cd/ml), and non-smokers (CdB, 0.2-0.6 ng Cd/ml), respectively [mean ± SE (n)]. However, effects of smoking cessation, measured longitudinally in each subject, did depend upon Cd exposure. During 4 days of smoking cessation, FOM CaU decreased in lower-Cd smokers (−16±5 mg Ca), showed no change in higher-Cd smokers (3±4 mg Ca), and increased in non-smokers (32±14 mg Ca). These results indicate that higher CdB values maintained elevated levels of urine calcium, even during a short period of smoking cessation. Results demonstrate that the Cd component of cigarettes may maintain elevated calcium excretion by the kidney, which may play a direct role in osteoporosis.

PS 278 CHRONIC EXPOSURE TO PARTICULATE CHROMATE INDUCES NUMERICAL CHROMOSOME INSTABILITY AND CENTROSOME AMPLIFICATION IN HUMAN LUNG CELLS.

A. L. Holmes[1, 2, 3], S. S. Wise[1, 2, 3], S. Pelsue[2, 3], W. Lingle[4], J. Salisbury[4] and J. P. Wise[1, 2, 3]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME,* [2]*Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME,* [3]*Department of Applied Medical Science, University of Southern Maine, Portland, ME and* [4]*Mayo Clinic and Foundation, Rochester, MN.*

Particulate hexavalent chromium (Cr(VI)) is a well-known human lung carcinogen; however, the carcinogenic mechanism remains unknown. Epidemiological and whole animal studies pinpoint particulate zinc chromate as the most potent Cr(VI) carcinogen; however, few studies have investigated its carcinogenic mechanism. Therefore, the purpose of this study was to determine if zinc chromate induces chromosome instability (CIN) and centrosome amplification in human lung cells. Exposure to zinc chromate for 24 h did not induce aneuploidy, but more chronic exposures induced a concentration- and time-dependent increase in aneuploidy. Consistent with the aneuploidy data, only longer exposures induced concentration- and time-dependent increases in centrosome amplification in both interphase and mitotic cells. In order to determine the mechanism of centrosome amplification, we investigated the number of centrioles in each centrosome and found that cells with amplified centrosomes exhibited centrosomes with normal centriole pairs and centrosomes with centriolar defects. These data indicate that multiple mechanisms are involved in zinc chromate-induced centrosome amplification. Lastly, we observed a prolonged G2 arrest after chronic exposure to zinc chromate which may play a role in the induction of centrosome amplification. All together, these data indicate that chronic exposure to zinc chromate induces CIN via centrosome amplification in human lung cells. This work was supported by NIEHS grant ES016893 (J.P.W.), cooperative agreement #EP-08-01 through the Maine Space Grant Consortium (J.P.W.), EPA GRO Fellowship MA-91685401 (A.L.H.) and the Maine Center for Toxicology and Environmental Health at the University of Southern Maine.

PS 279 BOWHEAD WHALE LUNG CELLS ARE RESISTANT TO CR(IV)-INDUCED GENOTOXICITY.

J. Martino[1, 2], S. S. Wise[1, 2], A. L. Holmes[1, 2], H. Xie[1, 2, 3] and J. P. Wise[1, 2, 3]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME,* [2]*Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME and* [3]*Department of Applied Medical Science, University of Southern Maine, Portland, ME.*

Particulate hexavalent chromium [Cr(VI)] is a recognized human lung carcinogen. It induces a spectrum of DNA damage including chromosome aberrations, DNA strand breaks and Cr-DNA adducts, which are thought to underlie its carcinogenic mechanisms. We are currently investigating the effects of Cr(VI) in marine mammals. Marine mammals are considered sentinels of ocean and human health, and a number of species are being used as models to study human diseases. Accordingly, we compared the cytotoxicity and genotoxicity of soluble and particulate Cr(VI) compounds in human and bowhead whale lung cells. We used the clonogenic survival assay to test cytotoxicity and the chromosome aberration, gamma-H2AX immunofluorescence and comet assays to assess genotoxicity. We also measured the intracellular uptake of chromium. Our data show that sodium chromate, zinc chromate and lead chromate were more cytotoxic to bowhead whale lung cells than to human cells. However, these compounds did not cause chromosome aberrations or DNA double strand breaks in bowhead whale lung cells. Activation of gamma-H2AX and lack of metaphases at the high doses suggests that Cr(VI) is affecting the whale cells, but it is unclear what effect underlies these outcomes. Currently, we are evaluating mechanisms that may explain the difference in response to Cr(VI) in human and whale cells, considering potential differences in DNA repair, cell death pathways, intracellular metabolism and chromatin remodeling. This work was supported by NIEHS grant ES016893 (J.P.W.), the Maine Center for Toxicology and Environmental Health at the University of Southern Maine, a Fulbright Grant (J.M.) and a Delta Kappa Gamma Society International World Fellowship (J.M.).

PS 280 THE IMPACT OF ALTERED GRAVITY ON CHROMATE GENOTOXICITY: USING A BIOREACTOR TO SIMULATE MICROGRAVITY IN CELL CULTURE.

J. P. Wise Jr.[1, 2], C. Wheeler[1, 2] and J. P. Wise Sr.[1, 2, 3]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME, [2]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME and [3]Department of Applied Medical Science, University of Southern Maine, Portland, ME.*

For the last two years, our lab has participated in NASA's Reduced Gravity Student Flight Opportunities Program. This program is designed to provide undergraduate students with the unique opportunity to conduct experiments on NASA's Weightless Wonder, a plane that simulates environments of microgravity and hypergravity by flying in a series of parabolas. We hypothesized that altered gravity (the combination of microgravity and hypergravity) would increase the amount of chromosomal damage induced by hexavalent chromium. To determine this, we treated human lung fibroblast cells with sodium chromate an hour before flight and stopped the exposure an hour after flight, for a total of four hours. Our data showed an increase of 2-3 times as much chromosomal damage, but about half as much chromium was getting into the cells as compared to ground controls. The next step is to determine what is causing this difference – hypergravity or microgravity. We are currently developing methods for this research using a centrifuge to simulate hypergravity, and a bioreactor to simulate microgravity. Our data from the bioreactor shows that there is a decrease in the amount of chromate-induced chromosomal damage as we decrease the speed of the bioreactor's rotation. Similarly, we see a decrease in the amount of chromium getting into the cells as the bioreactor's rotation speed decreases. The slower the rotation, the less gravity is on the cells; i.e. 23 rpm is 0.01431 g, and 5 rpm is 0.00089 g. These data suggest that altered gravity does indeed affect chromate-induced genotoxicity This work was supported by NASA grant ACD FSB-2009 (JPWSr), a fellowship from the Maine Space Grant Consortium (JPWJr) and the Maine Center for Toxicology and Environmental Health.

PS 281 GENOTOXIC EFFECTS OF DEPLETED URANIUM IN EXPOSED GULF WAR VETERANS WITH EMBEDDED FRAGMENTS.

M. McDiarmid[1], R. Albertini[2], M. Oliver[1] and K. Squibb[1]. *[1]University of Maryland School of Medicine, Baltimore, MD and [2]University of Vermont School of Medicine, Burlington, VT.*

In vitro and in vivo animal studies show evidence that uranium (U) is capable of causing genotoxicity due to its radioactive and chemical characteristics. For this reason, an on-going biennial medical surveillance program established by the Department of Veterans Affairs for U.S. soldiers exposed to depleted uranium (DU) during the 1991 Gulf War includes biomarkers of genotoxicity. Soldiers were exposed by inhalation, ingestion and wound contamination to small particles of DU oxides created when DU munitions impacted tanks with protective DU armor. Some soldiers also have embedded DU fragments in muscle tissue which serve as a constant source of systemic exposure to DU as evidenced by clinically elevated urine U (uU) excretion. Evidence of genotoxicity in peripheral blood collected from 35 members of the DU-exposed cohort was determined by analyzing for HPRT and PIGA mutations and for chromosomal aberrations in chromosomes 5, 7, 11 and 13 using fluorescent in-situ hybridization (FISH). Mean chromosomal aberrations in individuals with chronically elevated uU concentrations were not significantly different from those with normal uU concentrations when compared using Mann Whitney analysis. Mean mutation frequencies for HPRT and PIGA mutations were also not significantly different between the high vs low uU groups, however Poisson regression analysis indicated a significant ($p<0.01$) relationship between uU concentration and the frequency of HPRT mutations when the relationship was controlled for smoking, age and exposure to xrays. Significant relationships were not observed, however, when uU concentrations and the frequency of PIGA mutations or chromosomal aberrations were modeled by regression analysis. Thus, weak genotoxic effects of DU were evident only for one measure of mutation frequency and only in one type of statistical analysis. Mutational spectral analysis is planned to examine whether T cell clonality may be producing the increase in HPRT mutations. *This work was supported by the Department of Veterans Affairs.*

PS 282 PARTICULATE DEPLETED URANIUM INDUCES SELECTIVE X-CHROMOSOME FRAGMENTATION DEPENDENT ON XRCC3 IN CHINESE HAMSTER OVARY CELLS.

K. Joyce[1, 2], C. LaCerte[1, 2] and J. P. Wise[1, 2, 3]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME, [2]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME and [3]Department of Applied Medical Sciences, University of Southern Maine, Portland, ME.*

Depleted uranium (DU) is increasingly being used in industry and for military applications. The potential for both civilian and military personnel to be exposed to DU is rising, but there is limited research on the potential health hazards of DU exposure. Previous laboratory research has shown DU to be a potential carcinogen, but epidemiological studies have been inconclusive. In our study we used Chinese hamster ovary (CHO) cells to investigate the genotoxic effects of DU in wild-type, repair-deficient, and complemented CHO cell lines. The data show that 24 h particulate DU treatment (0, 0.5, 5, and 10 μg/cm2) induces concentration-dependent selective X-chromosome fragmentation in 0, 1, 27, and 44 percent of metaphases in wild-type (AA8) CHO cells, respectively. In XRCC3-deficient CHO cells (irs1SF), X-fragmentation was reduced to 0, 1, 5, and 4 percent of metaphases, respectively. The complimented cell line restored fragmentation levels to 0, 6, 36, and 49 percent, respectively. In addition we showed that 24 h particulate DU treatment (0, 0.5, 5, and 10 μg/cm2) induces concentration-dependent selective X-chromosome fragmentation in wild-type (51D1 lox), Rad51D-deficient (51D1), and Rad51D-complimented (51D1.3) cell lines, but fragmentation frequency is not impacted by Rad51D deficiency. Future work will determine time-dependence and examine additional repair-deficient CHO lines. This work was supported by ARO Grant #W911NF-04-1-0240 (J.P.W.) and ARO Grant #W911NF-09-1-0296 (J.P.W.), and the Maine Center for Toxicology and Environmental Health.

PS 283 EFFECTS OF DEPLETED URANIUM ON ETOPOSIDE-INDUCED DNA DAMAGE AND REPAIR IN HUMAN BRONCHIAL EPITHELIAL CELLS.

M. Yellowhair[1] and D. Lantz[2]. *[1]Pharmacology & Toxicology, The University of Arizona, Tucson, AZ and [2]Cell Biology & Anatomy, The University of Arizona, Tucson, AZ.*

It has been widely reported that metals can be genotoxic by several mechanisms including generation of reactive oxygen species and inhibition of DNA repair. There is an increased concern over environmental exposure to abandoned uranium mine tailings as well as occupational exposures to depleted uranium via military action. The aim of this study is to evaluate the ability of depleted uranium (DU) to inhibit double strand break DNA repair at low concentrations in human bronchial epithelial (16HBE14o-) cells. 16HBE14o- cells were co-exposed to 0.13 – 200 μM of soluble depleted uranium as uranyl acetate (UA) in the presence of 0 – 25 μM of etoposide for 0 – 48 hr. Cytotoxicity was assessed in vitro by clonogenic survival assay. DNA damage response was assessed in vitro via Western immunoblotting to determine phosphorylation of various DNA repair proteins. Results indicate that co-exposures of etoposide and UA induced significant synergistic cytotoxicity compared to individual treatments and untreated cells. 16HBE14o- cells treated with UA treatments did not induce phosphorylation of DNA repair proteins compared to etoposide treatments and untreated cells. These results suggest that DU may inhibit double strand break DNA repair at low concentrations in the presence of a known DNA damaging agent. The inhibition of DNA repair by depleted uranium at environmentally and physiologically relevant concentrations suggests a novel means by which uranium may exert its genotoxic effects. This work is supported by NIH Grants CA096281 (RCL), F31ES014971 (MY), P30ES006694, P42ES004940 and The Alfred P. Sloan Foundation (MY).

PS 284 THROMBOPOIETIN (TPO) AND ITS RECEPTOR (MPL) ARE INVOLVED IN MEGAKARYOPOIESIS STIMULATION AFTER VANADIUM PENTOXIDE INHALATION.

T. I. Fortoul, A. Gonzalez-Villalva, P. Diaz-Bech and P. Diaz-Fuentes. *Biologia Celular y Tisular, UNAM, Mexico City, Mexico.*

Previous work from our group has reported hematological changes, such anemia and thrombocytosis after inhaled vanadium exposure. Platelets and megakaryocytes increased at subchronic and chronic exposure. Also, we have found ultrastructural modifications, and JAK/STAT activation on megakaryocytes in exposed mice. The primary regulator of platelet production is Thrombopoietin (TPO) and it binds to

its receptor Mpl to exert its function. The aim of this study was to evaluate the presence of TPO in the liver and lung and the presence of the Mpl receptor on megakaryocytes. Twenty CD-1 male mice were exposed to vanadium pentoxide (V2O5) [0.02M] one hour twice a week during 8 weeks according to our model. Spleen, liver and lungs, were removed at fourth, sixth and eight-week exposure. A spleen section was smashed, centrifuged at 1500rpm/5min; erythrocytes were lysed. Cells were inmuno marked for CD41 as surface marker of megakaryocytes and for Mpl. Liver, lungs and the other fragment of the spleen were processed for paraffin embedding and then processed of inmunohistochemistry to detect TPO and Mpl. There were increases in TPO at all times of exposure on lung and liver. Mpl was found decreased at eight-week exposure by both methods. The results showed that TPO is involved on megakaryopoiesis induced by inhaled V and it is remarkable the presence of TPO in lung, because it has been proposed as a important site of megakaryopoiesis and the production of TPO has been associated to altered pulmonary function. We propose that the decrease in Mpl on megakaryocytes could be a negative JAK/STAT regulator pathway that is activated by vanadium.
Supported in part DGAPA-PAPIIT-IN210409

PS 285 NON-CILIATED BRONCHIOLAR CELL POPULATION CHANGES AFTER VANADIUM INHALATION.

C. I. Falcon-Rodriguez[1, 4], N. Lopez-Valdez[1, 4], S. Hernandez-Ojeda[2], J. Espinoza-Aguirre[2], T. I. Fortoul[1] and M. Altamirano-Lozano[3]. *[1]Biologia Celular y Tisular, UNAM, Mexico City, Mexico, [2]Departamento de Medicina Genómica y Toxicología Ambiental; Insituto de Investigaciones Biomedicas, UNAM, Mexico City, Mexico, [3]Unidad de Investigación en Genética y Toxicología ambiental, Bioterio, Campo-II, Facultad de Estudios Superiores Zaragoza, UNAM, Mexico City, Mexico and [4]Posgrado Ciencias Biológicas, UNAM, Mexico City, Mexico.*

Vanadium is adsorbed into carbon particles that enter into the organism and is deposited in bronchioles damaging Non-Ciliated Bronchiolar or Clara Cell (NCBC) that participates in bronchioles repair. CCSP protein is a NCBC marker and it is known to exhibit anti-inflammatory and immumodulatory functions. Some researchers found subpopulations of naphthalene-resistant NCBC express protein (CCSP); others, a variant that expresses both CCSP and SP-C; a phenotype change after antigen-exposed resulting in PAS-positive cells has been also reported. Because of its important function it is relevant to analyze its changes after vanadium inhalation. CD1 male mice were distributed in four groups: Control, 4, 6, and 12- week exposure to vanadium. Lungs were perfumed with saline via right cardiac ventricle. Fixed with 4% paraformaldehyde. Lungs were removed, embedded in paraffin and cut into longitudinal sections. NCBC were labeled using a rabbit-CCSP antibody and counterstained with PAS stain. Distal bronchiolar epithelium in exposed mice contained PAS-positive, mucin granules after a 6-week vanadium exposure not visible in controls, these changes suggest the presence of at least two NCBC populations in response to V exposure.
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PS 286 VANADIUM PENTOXIDE INHALATION EFFECT IN A MURINE MODEL OF LUNG CANCER.

N. Lopez-Valdez[1, 2], C. I. Falcon-Rodriguez[1, 2], M. Gonsebatt-Bonaparte[3], M. Altamirano-Lozano[4] and T. I. Fortoul[1]. *[1]Biologia Celular y Tisular, UNAM, Mexico City, Mexico, [2]Posgrado de Ciencias Biologicas, UNAM, Mexico City, Mexico City, Mexico, [3]Departamento de Toxicología y Medicina Genómica. Instituto de Investigaciones Biomédicas, UNAM, Mexico City, Mexico City, Mexico and [4]Unidad de Investigación en Genética y Toxicología Ambiental, Bioterio, Campo-II. Facultad de Estudios Superiores-Zaragoza, UNAM, Mexico City, Mexico City, Mexico.*

Lung cancer is one of the main causes of death worldwide. Atmospheric environmental carcinogens have been identified as an important cause in its etiology. In 2003, numerous environmental causes of lung cancer have been identified. This pathology has been associated with air pollution worsening, especially with particulate matter. These particles contain some trace elements like vanadium. Several animal studies have shown that vanadium is effective in preventing and/or reducing chemically induced preneoplastic and neoplastic grow in various target organs. Studies indicated that vanadium limits cell proliferation related molecules, like PCNA. In vitro assays showed V arrests Cell Cycle on S-phase by p53 activation. To know about vanadium pentoxide effect on a carcinogenesis model, we evaluated its consequences in a lung carcinogenesis model. CD-1 male mice were distributed in three groups: Control, Urethane and Urethane/ V2O5. Control mice inhaled saline 1hr/twice a week; Urethane mice were injected with 1mg/g bw; Urethane/ V2O5 group was injected with urethane and simultaneously inhaled V2O5 0.02M 1hr/twice a week. Urethane was used as carcinogenic agent. Animals were sacrificed by pentobarbital overdose at 8-week exposure time. Adenomas morphometric analysis was conducted to determine lung adenomas size, and inmunohistochemistry for PCNA was also performed. Inhalation of V2O5 produced size reduction and less tumors after an 8-week Urethane/V2O5 treatment; PCNA revealed a decrease in Urethane/V adenomas.This study indicates vanadium antiproliferative effect on urethane induced lung adenomas.
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PS 287 CHANGES IN SPLEEN LYMPHOCYTES PROLIFERATION PATTERN IN MALE MICE CAUSED BY V2O5 INHALATION.

V. Rodriguez-Lara[1], A. Muñiz Rivera-Cambas[2] and T. I. Fortoul[1]. *[1]Biologia Celular y Tisular, UNAM, Mexico City, Mexico and [2]Facultad de Ciencias, Biologia, UNAM, Mexico City, Mexico City, Mexico.*

Vanadium pentoxide (V2O5) is an air pollutant released to the atmosphere by the burning of fossil fuels. Vanadium exposure produces adverse systemic effects such as an increase in the number of megakaryocytes, neutrophils, monocytes, platelets and lymphocytes. Splenomegaly mainly attributed to an increase in the white pulp, which looses its normal histological conformation has been also observed. Lymphocytes within the white zone also have morphologically altered nucleus. Considering the implication that this finding may have, it is important to confirm that the main cause of the increase in lymphocytes number in the spleen is the consequence of proliferation. To confirm our hypothesis Ki-67 was used as a faithful marker of cells cycling. CD1 male mice 35g inhaled V2O5 [0.02M] one hour twice a week. Mice were sacrificed at 1, 4, 8, and 12 weeks; spleen was removed, fixed and processed for standard immunohistochemistry to Ki-67. Results show an increment in the percentage of lymphocytes marked with Ki-67 since the first week of exposure; this was seen in a time-dependent manner. The proliferative effect of vanadium on lymphocytes enhances the possibility of inducing neoplastic cells; this effect may be seen in other blood cells that are also increased in number. Likewise, lymphocytes in great amount can provoke a deregulation in the immune system and this may cause auto-reactive cells that trigger an autoimmune pathology.
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PS 288 MORPHO-PHYSIOLOGICAL ALTERATIONS IN LYMPH NODE DUE TO VANADIUM PENTOXIDE INHALATION.

L. S. Lopez-Zepeda and T. I. Fortoul. *Biologia Celular y Tisular, UNAM, Mexico City, Mexico.*

Previous studies in our group have shown several alterations in lymphoid organs due to vanadium pentoxide inhalation, such as proliferation and modifications in the actin cytoskeleton of spleen's lymphocytes. Vanadium has also been shown to increase the production of antibodies, however the specificity of these has been significantly reduced. All these findings were suggestive of a possible lymphocytic activation. Still, lymph nodes have not been studied using our model. Which is why we decided to asses whether the lymphocytes of the lymph node were activated or not. Fifteen CD-1 female mice were divided into two groups. Exposed mice inhaled vanadium pentoxide [0.02M] and controls saline for one hour twice a week. Their lymph nodes were obtained after 4 weeks of exposure, fixed with paraformaldehyde and embedded in paraffin. Immunofluorescence for CD25 was done knowing it as late activation marker, took photographs using a LEICA TCS-SP5 confocal microscope. A significant increase in the CD25 stain was observed, particularly higher in the lymph node medulla. This supports our hypothesis and leads us to investigate even further the mechanisms by which vanadium activates lymphocytes without an actual immune challenge.
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PS 289 FIRST STEPS TOWARDS AN UNDERSTANDING OF A MODE OF CARCINOGENIC ACTION FOR VANADIUM PENTOXIDE.

D. McGregor[1], D. Schuler[2], K. Morgenthal[2], H. J. Chevalier[3], M. Merker[4], J. L. Ravanat[5], P. Sagelsdorf[2], M. Walter[2] and K. Weber[2]. *[1]Toxicity Evaluation Consultants, Aberdour, United Kingdom, [2]Harlan Laboratories Ltd., Füllinsdorf, Switzerland, [3]AnaPath GmbH, Oberbuchsiten, Switzerland, [4]Harlan Cytotest Cell Research GmbH, Rossdorf, Germany and [5]INAC/SCIB/LAN, CEA-Grenoble, Grenoble, France.*

In NTP 2-year whole-body exposure studies, vanadium pentoxide increased the incidence of alveolar/bronchiolar neoplasms to about 80% at all concentrations tested (1, 2 or 4 mg/m3) in B6C3F1 mice, whereas the lung tumour responses in F344/N rats were, at most, ambiguous. No neoplastic response occurred elsewhere, including other sites of first contact, whereas non-neoplastic effects were recorded

in nose, larynx and lungs and showed a more usual exposure-related response in both species. Published studies suggest vanadium pentoxide is mutagenic in vitro and possibly in vivo in mice, but this does not explain the species or site specificity of the neoplastic response. As a first step in an investigation of this problem, a nose-only inhalation study was conducted in female B6C3F1 mice at vanadium pentoxide concentrations of 0, 0.25, 1 and 4 mg/m3, 6h/day for 16 days. Observations included atmospheric V, concentrations of V in lungs and blood, lung weight, histopathology of the airways, cell proliferation in lungs, concentrations in lungs of α-tocopherol, glutathione (reduced and total), F2-isoprostane and 9 specific DNA-oxy-adducts and DNA damage (comet assays) in lung and BAL fluid cells.
No effects were observed at 0.25 mg/m3. At 1 and 4 mg/m3, exposure-dependent increases were observed in lung weight, alveolar histiocytosis, sub-acute alveolitis and/or granulocytic infiltration and a generally time-dependent increased cell proliferation rate. No DNA damage (comet) was detected and the only DNA oxy-adducts at or above the limit of detection were 8-oxodGuo and dCyd341, but only 8-oxodGuo showed a dose related increase.
This work was conducted in collaboration with Advanced Technology Institute, Charleston, SC 29418, USA and sponsored by the US Army Research Laboratory under Cooperative Agreement Number DAAD 19-03-2-0036.

PS 290 TISSUE BURDEN AND HISTOPATHOLOGY IN SPRAGUE-DAWLEY RATS AFTER INHALATION OF TUNGSTEN ALLOY.

P. A. Ortiz[1], C. U. Parkinson[2], V. P. Mokashi[1], M. G. Stockelman[1] and B. A. Wong[1, 2]. *[1]Naval Medical Research Unit - Dayton, Wright-Patterson AFB, OH and [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Tungsten is a transition metal element with unique physicochemical properties that have been used for a variety of military, medical, and industrial purposes. Tungsten has largely replaced lead in high kinetic energy penetrators, small caliber ammunition, and other U.S. military munitions. In addition, it has also replaced depleted uranium in armor. These uses present an exposure risk to military personnel from aerosolized metal following weapons firing or ballistic impact. Tungsten is typically mixed with other metals such as nickel or cobalt to provide an alloy with strength and ductility. The objective of this study was to determine the translocation and accumulation of tungsten, nickel and cobalt in tissues of rats after inhalation of tungsten metal alloy particles. To accomplish this, male Sprague Dawley rats underwent a 2 week exposure to either air or tungsten alloy (approximately 5 mg/m^3, 6 h/d, 5 d/wk, for 2 weeks). Animals were held for up to 21 days post-exposure to examine the distribution of those metals. Animal body weights were not affected by the tungsten alloy inhalation. Tissues were analyzed for tungsten alloy burden using neutron activation analysis and histopathology. All three metals (tungsten, nickel and cobalt) were detected in the lung. However, cobalt was the only metal detected in other tissues, including kidney, liver and olfactory bulb. These results imply that cobalt behaved like a soluble compound while tungsten results are consistent with insoluble metal particles. Finally, there were no gross pathological observations detected in the tissues. Our data indicate that the alloy plays no role in the distribution of tungsten, including by olfactory translocation.

PS 291 EFFECTS OF TUNGSTEN CHEMICAL SPECIES ON PHOSPHATE-DEPENDENT PATHWAYS IN AN OSTEOBLAST CELL LINE.

D. R. Johnson[1] and C. Ang[2]. *[1]Environmental Laboratory, U.S. Army Engineer Research & Development Center, Vicksburg, MS and [2]Badger Testing Services, Vicksburg, MS.*

Tungsten (W) is a metal that has numerous civil and military applications due to its high strength and melting point. When W enters the environment, it is rapidly oxidized and speciated based on the environmental matrix it is embedded in. Bone is the long-term storage organ for W—and presumably W chemical species if present—when taken up by organisms. It is unknown what long-term effects W has in bone. Extensive polymerization of W to phosphate may deplete intracellular phosphate stores, disrupting phosphorylation reactions in cells. Furthermore, disruption of normal osteoblast function by W may impact bone formation and biomechanics. Therefore, we evaluated the effects of W species on several phosphate-dependent intracellular functions, including energy cycling (ATP production), regulation of enzyme activity (protein tyrosine kinase [PTK]), and intracellular secondary messengers (cyclic adenosine monophosphate [cAMP]). hFOB 1.19 osteoblastic cells were exposed to vehicle control (water) or W chemical species (sodium tungstate, phosphotungstate [PW], poly tungstate [polyW], and tungstosilicic acid [TSA]) at 0-10,000 μM for 24 h at 37 degrees C. Cells were then lysed and analyzed for cell viability, ATP concentration, tyrosine kinase activity, and cAMP concentration. W species did not affect ATP concentrations except at the highest concentration. PW, polyW, and TSA, but not tungstate, significantly increased protein tyrosine kinase activity at 10,000 μM after 4 h exposure. Tungstate and PT increased cellular cAMP at ≥ 100 μM, while TSA decreased cellular cAMP at 10,000 μM. These data demonstrate that W chemical species generally only affect phosphate-dependent cell signaling and secondary messenger pathways in hFOB 1.19 cells. However, the W chemical species concentrations needed to affect these phosphate-dependent biochemical pathways greatly exceed the amount of W deposited in bone (100 μM W = 18 mg/kg), thus providing evidence that W chemical species will not affect osteoblastic cellular activity.

PS 292 THE INFLUENCE OF GENETIC POLYMORPHISMS ON MERCURY BIOMARKER DISTRIBUTION IN MEXICAN MOTHER-CHILD PAIRS.

J. Goodrich[1], D. Cantonwine[1], B. N. Sánchez[2], M. Hernández-Avila[3], H. Hu[1], M. M. Téllez-Rojo[4] and N. Basu[1]. *[1]Environmental Health Sciences, University of Michigan, Ann Arbor, MI, [2]Biostatistics, University of Michigan, Ann Arbor, MI, [3]Ministry of Health, México, Districto Federal, Mexico and [4]Statistics, Center for Evaluation Research and Surveys, National Institute of Public Health, Cuermavaca, Morelos, Mexico.*

Mercury (Hg) risk assessment is complicated by variability in toxicokinetic parameters and distribution to established biomarkers of exposure (blood, hair, urine). This work hypothesizes that single nucleotide polymorphisms (SNPs) in key glutathione S-transferase (GST), glutathione synthesizing, and selenoprotein genes underlie inter-individual differences in Hg biomarker levels. The genotype of 17 SNPs and biomarker Hg levels were obtained from mother-child pairs of the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) cohort. Mothers' hair and children's urine Hg levels (n=385; mean±SD=0.53±0.48 μg/g; n=440; 0.96±2.97 μg/L) mirrored US population averages of similar groups. Indicating higher methylmercury exposure, blood (n=415; 1.65±1.3 μg/L) and hair (n=444; 0.5±0.46 μg/g) Hg biomarker levels in the children were 2-4 times higher than that of US children. Mean biomarker Hg levels and biomarker ratios (hair:blood, blood:urine) were compared across genotype groups using analysis of variance. Initial analyses suggest that several SNPs (in GSTP1, GSTM3, GCLC, SEPP1, GPX2) may influence accumulation of Hg and its distribution among the biomarkers. Linear regression modeling will further explore these relationships. In conclusion, genetic polymorphisms may modify the distribution of Hg to blood, hair and urine. Such gene-environment studies may help to improve our understanding of the mechanisms underscoring mercury distribution in the body and minimize variation associated with common exposure biomarkers.

PS 293 LEAD CAUSES BONE LOSS BY DEPRESSION OF OSTEOBLASTIC FUNCTION THROUGH INHIBITION OF WNT SIGNALING.

E. E. Beier[1, 2], E. Puzas[1, 2], M. Zuscik[2], D. Cory-Slechta[1] and T. Sheu[2]. *[1]Environmental Medicine, University of Rochester, Rochester, NY and [2]Orthopedics, University of Rochester, Rochester, NY.*

INTRODUCTION: We postulate that Pb exposure causes a weakening of the skeletal structure that leads to an accelerated onset of osteoporotic-like reduction in bone mineral density. We have found that Pb inhibits the ability of osteoblasts to make bone while observing the novel induction of the SOST gene. SOST encodes for the protein sclerostin, a potent repressor of the anabolic Wnt pathway in bone. Thus, our central hypothesis is that Pb causes bone loss by suppressing osteoblast function, and that this primarily occurs through induction of sclerostin and depression of Wnt signaling. METHODS: Rat osteoblasts were isolated from neonatal rat calvaria and were used to test the influence of Pb on bone nodule formation. Female Long-Evans rats were given lifelong exposure to 50 ppm Pb and at 18 months were sacrificed and skeletal elements were harvested. Bone parameters were measured by histomorphometry and μCT. Strength testing was performed on vertebral specimens by compression to failure. Immunohistochemistry was assessed using antibodies for β-catenin and sclerostin. RESULTS: Pb dose dependently decreased bone nodule formation, which was paralleled by increased sclerostin levels. Wnt3a offered a partial remediation of the Pb effect. We also observed that Wnt3a induced β-catenin activation was blunted at 12 hours only with a 48 hour Pb pretreatment. Rats exposed to Pb (9.16 μg/dL) had systemically lower bone density compared to water controls. They also had weaker bones that were quicker to fracture and displayed depressed β-catenin signaling. DISCUSSION: Our results indicate that Pb inhibits osteoblastic activity. Pb exposure evokes significant changes consistent with a low bone mass phenotype at continuous blood levels below 10 μg/dl. These studies are the first direct evidence identifying the inhibition of Pb on a key signaling pathway providing insights into a molecular mechanism underlying Pb toxicity in the skeleton.

PS 294 LOW LEVEL OF LEAD CAN INDUCE PHOSPHATIDYLSERINE-EXPOSURE AND ERYTHROPHAGOCYTOSIS: A NEW MECHANISM UNDERLYING LEAD-ASSOCIATED ANEMIA.

J. Noh, K. Kim, W. Jang, K. Lim, S. Kang, O. Bae and J. Chung. *College of Pharmacy, Seoul National University, Seoul, Republic of Korea.*

Anemia is probably one of the most well-known toxic effects of lead. Previously, lead-induced anemia was considered to be from the inhibition of δ-aminolevulinic cid dehydratase (ALAD) participating in the heme iosynthesis. However, it was not known whether lead could ffect the destruction of erythrocyte, another important actor for anemia. In the present study, we demonstrated hat lead could accelerate the splenic sequestration of rythrocytes through phosphatidylserine (PS) exposure and ubsequently increased erythrophagocytosis. In freshly solated human erythrocytes, Pb^{2+} induced PS exposure at relatively low concentrations (~0.5 μM) by inhibiting flippase, a key aminophospholipid translocase for the maintenance of PS asymmetry and ATP depletion appeared to underlie this phenomenon. Abnormal shape changes of erythrocytes and microvesicle generation, another trigger for the erythrophagocytosis were also observed in the Pb^{2+}-exposed erythrocytes. In vitro data showed that human macrophage indeed recognized and phagocytosis PS-exposed erythrocytes. In a good accordance with these in vitro results, the oral administration of Pb^{2+} increased PS exposure on erythrocytes in rat in vivo. In addition, reduction of hematocrit and hemoglobin and increased spleen weight were observed along with enhanced splenic sequestration of erythrocytes in the rats subchronically exposed to Pb^{2+} through drinking water. In conclusion, these results suggest that Pb^{2+}-induced anemia may be explained at least in part by increased PS exposure on erythrocytes, erythrophagocytosis and splenic sequestration.

PS 295 ACUTE ORAL LEAD BULLET FRAGMENT EXPOSURE IN NORTHERN BOBWHITE QUAIL.

R. Kerr[1], J. Hollady[1], T. Jarrett[1], S. D. Holladay[1], B. Selcer[1], B. Meldrum[2], S. Williams[3], L. Tannenbaum[4] and R. M. Gogal[1]. [1]*Department of Anatomy and Radiology, University of Georgia, Athens, GA,* [2]*Department of Biomedical Sciences & Pathobiology, Virginia Tech, Blacksburg, VA,* [3]*Poultry Diagnostic & Research Center, University of Georgia, Athens, GA and* [4]*U.S. Army Center for Health Promotion & Preventive Medicine, Department of Defense, Aberdeen, MD.*

Lead (Pb) is a worldwide environmental contaminant known to adversely affect multiple organ systems in mammalian and avian species. In birds, the primary route of exposure is by oral ingestion of lead particles as grit or mistakenly as food. Current data are lacking in the retention and clearance of Pb bullet fragments in the bird's GI tract while linking toxicity with blood Pb levels. In the present study, Northern Bobwhite quail, fed a seed-based diet, were orally gavaged with Pb bullet fragments (0, 1 and 5 fragments/bird) and evaluated for rate of fragment clearance, changes in peripheral blood, renal, immune and gastrointestinal parameters. Based on radiographs, the majority of the birds cleared or absorbed the fragments by 7 days with the exception of one 5-fragment bird, which took between 7-14 days. Blood lead levels were higher in the males than females. Feed consumption, body weight gain, packed cell volume (PCV), plasma protein concentration, δ-aminolevulinic acid dehydratase (d-ALAD) activity were adversely affected by oral gavage with 5 fragments in the males. However, Koilin degradation was observed only in the 5 fragment female birds. Birds receiving a single lead pellet displayed depressed d-ALAD suggesting altered hematologic function while birds acutely exposed to 5-bullet fragments exhibited greater morbidity.

PS 296 ORAL LEAD PELLET EXPOSURE IN NORTHERN BOBWHITE QUAIL: A 56-DAY STUDY.

R. M. Gogal[1], R. Kerr[1], J. Holladay[1], S. D. Holladay[1], L. Tannenbaum[2], B. Meldrum[3] and S. Williams[4]. [1]*Department of Anatomy and Radiology, University of Georgia, Athens, GA,* [2]*Department of Biomedical Sciences and Pathobiology, Virginia Maryland Regional College, Blacksburg, VA,* [3]*U.S. Army Center for Health Promotion and Preventative Medicine, Department of Defense, Aberdeen, MD and* [4]*Poultry Diagnostic and Research Center, University of Georgia, Athens, GA.*

We recently reported in a 28 day study that acute oral lead (Pb) pellet (#9 shot) exposure in Northern Bobwhite quail resulted in significant toxicity in birds ingesting 5 Pb pellets or greater. Birds that received a single lead pellet showed no overt clinical signs of toxicity, but had sustained depressed plasma δ-aminolevulinic acid dehydratase (d-ALAD) activity. In the present study, bobwhite quail, fed the same seed-based diet, were orally gavaged once with Pb spent shot (0, 1, 2 and 3 pellets/bird) and evaluated for 56 days for changes in feed consumption, weight gain, peripheral blood Pb levels and physiology, renal, immune and gastrointestinal parameters. Birds in the 3 pellet group showed a significant decrease in weight gain which did not correlate with feed consumption. Mean blood Pb levels increased proportionately to the number of pellets ingested at 7 days post exposure. Similar to the previous study, male blood lead levels were significantly higher compared to the females up to 14 days post exposure. These Pb blood levels declined weekly but remained elevated throughout the study according to baseline data. Similar to the previous study, the δ-aminolevulinic acid dehydratase (d-ALAD) activity decreased significantly in all Pb pellet-treated birds, began to recover in the males but remained lower than controls. Blood hematology, immune cytology and the other endpoints were not remarkable. The results of this study support our previous findings with regards to the 1 pellet birds and extend it by showing that birds exposed to 2 and 3 pellets, although becoming transiently moribund, began to show clinical signs of recovery 2 months post exposure.

PS 297 ALUMINIUM TOXICITY AND ELEMENTAL COMPOSITION CHANGES IN *CAENORHABDITIS ELEGANS.*

K. E. Page[1, 2], D. W. Killilea[3], K. N. White[2], C. R. McCrohan[2] and G. J. Lithgow[1]. [1]*The Buck Institute for Age Research, Novato, CA,* [2]*Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom and* [3]*Children's Hospital Oakland Research Institute, Oakland, CA.*

Aluminium (Al) is a highly abundant crustal metal with known toxic effects in multiple biological systems. The free-living terrestrial nematode *Caenorhabditis elegans* (*C. elegans*) is widely used for toxicity and aging studies due to its small size, large progeny numbers, short lifespan, and simple methods of genetic manipulation. This makes *C. elegans* a good model to study the toxicity mechanism of Al in animals. Aluminium exposures were carried out on agar plates with Al in the form of $Al(NO_3)_3$. Exposure of the nematode to Al results in altered elemental composition of the organism with severe detrimental effects. Aluminium exposure alters the course of normal aging in *C. elegans* from 1.9 mM. Aluminium negatively affects *C. elegans* developmental progression from 3 μM Al, fertility at 30 μM Al, and body size from 1.9 mM Al. The developmental delay phenotype caused by Al exposure was observed even when exposure only occurred during embryogenesis. This transgenerational toxic response poses questions concerning in utero Al exposure in humans. At 4.8mM Al, the levels of not only Al, but a multitude of other elements in the worms are altered. We have also found a set of genetic modulators of Al toxicity. We have also found a set of genetic modulators of Al toxicity. These genes have potential relevance in the metallostasis (metal homeostasis) of other metals, and a sub set are key regulators of known stress resistance pathways. Knock-down of these gene products alter the elemental profile of the worm. This provides a potential mechanism of action for these genes in Al toxicity and general stress response.

PS 298 BIOACCESSIBILITY-BASED READ-ACROSS ASSESSMENT OF NICKEL COMPOUNDS FOR ORAL SYSTEMIC TOXICITY.

R. G. Henderson[1], D. Cappellini[2], S. Seilkop[3], A. R. Oller[1] and H. K. Bates[1]. [1]*NiPERA, Inc., Durham, NC,* [2]*Kirby Memorial Health Center, Wilkes-Barre, PA and* [3]*SKS Consulting Services, Siler City, NC.*

A read-across assessment for oral systemic toxicity was undertaken for a series of nickel (Ni) substances. Bioaccessibility can provide information regarding potential bioavailability and subsequent toxicity such as acute and chronic oral toxicity and reproductive toxicity. Twelve Ni-containing samples were extracted in synthetic gastric and intestinal fluids. Acute oral toxicity studies in rats were conducted with eleven of these substances to verify that the bioaccessibility data provide reasonable estimates of systemic bioavailability and hence toxicity. The oral LD_{50} data were well predicted by Ni release data for each substance (R^2=0.91). For substances with existing oral LD_{50} data, results were compared with their respective EU hazard classification. For substances without LD_{50} data, one sided lower confidence limits for predicted LD_{50} values were calculated. Results indicated with 95% confidence that samples releasing <48% available Ni (mg Ni released/mg available Ni x 100) are predicted to have an LD_{50} >2000 mg substance/kg; samples releasing >76% available Ni are expected to have an LD_{50} between 200-300 mg substance/kg; and samples with 48-76% bioaccessibility are predicted to have an LD_{50} between 300-2000 mg substance/kg. When Ni substances were allocated into these three groups according to bioaccessibility, the hazard classifications for systemic oral toxicity were determined based on read-across from three data-rich nickel compounds. Samples

releasing <48% available nickel were read-across from Ni oxide and Ni subsulfide. Samples releasing >76% were read-across from Ni sulfate. No source or target substances released 48-76% available Ni in this study. Therefore, in the absence of additional verification data, read-across from the more conservative source substance (i.e., Ni sulfate) was recommended. Substances read-across from Ni sulfate warrant classification for acute (Xn;R22 and Acute Tox. 3) and reproductive (Cat 2;R61 and Repro 1B) toxicity in the EU.

PS 299 NICKEL(II) CHLORIDE CAUSES PANCREATIC ISLET β-CELL DEATH *VIA* A JNK-REGULATED MITOCHONDRIAL APOPTOTIC PATHWAY.

D. Hung[2, 1], K. Chen[3], H. Wu[3], Y. Chen[4], C. Su[5], C. Yen[6], T. Lu[1], Y. Yang[1], T. Ho[7] and C. Huang[7]. *[1]Graduate Institute of Drug Safety, China Medical University, Taichung City, Taiwan, [2]Toxicology Center, China Medical University Hospital, Taichung, Taiwan, [3]Department of Urology, China Medical University Hospital, Taichung, Taiwan, [4]Department of Physiology, China Medical University, Taichung, Taiwan, [5]Department of Otorhinolaryngology, Head and Neck Surgery, Changhua Christian Hospital, Changhua, Taiwan, [6]Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, Taiwan and [7]School of Chinese Medicine, China Medical University, Taichung, Taiwan.*

Nickel is a toxic heavy metal, which is widely used in electroplating and alloys producing. Previous studies have shown that long term exposure to nickel would lead intoxication in several organs, such as pneumonitis, rhinitis, sinusitis, dermatitis, nasal cavity and lung cancers. Several studies have shown that nickel possesses the ability to induce hyperglycemia. However, the precise action and mechanism of nickel on the growth and function of pancreatic islet β-cells are still unclear. Thus, the present study is designed to investigate the effects and possible mechanisms of nickel chloride (NiCl2) exposure on islet β-cell line RIN-m5F cells. The results showed that the cell morphology of RIN-m5F cells was significantly changed 24 hours after treatment with NiCl2. The flow cytometric analysis showed that the sub-G1 cell count and Annexin V-Cy3 fluorescence were dramatically increased in NiCl2-treated RIN-m5F cells. Apoptosis-related caspases including caspase-3, caspase-7, and caspase-9, were also activated in NiCl2-treated RIN-m5F cells. Furthermore, NiCl2 could also significantly enhance the mRNA expressions of p53, Bid, and Bak. Besides, NiCl2 increased cytochrome c release from mitochodria to cytosol. The mitochondrial transmembrane potential was also decreased by NiCl2. These NiCl2-induced effects could be reversed by JNK inhibitor SP600125. Taken together, these results indicate that exposure to NiCl2 induces the cytotoxicity and apoptosis in pancreatic islet β-cells via a JNK-regulated mitochondrial apoptotic pathway.

PS 300 LOW-DOSE PYRROLIDINE DITHIOCARBAMATE/COPPER COMPLEX INDUCES LUNG EPITHELIAL CELL APOPTOSIS *VIA* THE ER-STRESS-RELATED SIGNALING PATHWAYS.

Y. Chen[1], K. Chen[2], C. Chen[3, 4], H. Wu[2], C. Su[5], D. Hung[6, 4], C. Yen[7], Y. Yang[4] and T. Lu[4]. *[1]Department of Physiology, China Medical University, Taichung, Taiwan, [2]Department of Urology, China Medical University Hospital, Taichung, Taiwan, [3]Department of Emergency, China Medical University Hospital, Taichung, Taiwan, [4]Graduate Institute of Drug Safety, China Medical University, Taichung, Taiwan, [5]Department of Otorhinolaryngology, Head and Neck Surgery, Changhua Christian Hospital, Changhua, Taiwan, [6]Toxicology Center, China Medical University Hospital, Taichung, Taiwan and [7]Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, Taiwan.*

Pyrrolidine dithiocarbamate (PDTC) is widely used in pesticides, fungicides, insecticides, and herbicides. Copper (Cu) is a toxic heavy metal in the environment, and an essential trace metal element in the body, which is involved in many biological processes as a catalytic cofactor. The present study is designed to investigate the cellular toxicity of low-dose PDTC, CuCl2, and PDTC/Cu complex exposure in lung alveolar epithelial cells that serve primary structural and functional roles in the lungs. The results showed that PDTC or CuCl2 alone did not affect cell viability, but PDTC/Cu complex significantly decreased lung alveolar epithelial cell viability. PDTC/Cu complex dramatically enhanced the phosphorylations of JNK and ERK proteins. PDTC/Cu complex was capable of activating the apoptosis-related caspases including caspase-9, caspase-7, and caspase-3, which could be reversed by the addition of JNK inhibitor SP600125 or transfection of MAPK8 short hairpin RNA. PDTC/Cu complex also induced mitochondrial dysfunction. Furthermore, PDTC/Cu complex could trigger the expressions of ER stress-associated signaling molecules including Grp78, Grp94, caspase-12, ATF4, and CHOP, which could be reversed by SP600125. Taken together, these results indicate that exposure to low-dose PDTC/Cu complex induces cytotoxicity and apoptosis in alveolar epithelial cells via the ER-stress-related signaling pathways.

PS 301 CHEMICAL FORM OF METALS IN TRADITIONAL MEDICINES UNDERLINES POTENTIAL TOXICITY IN CELL CULTURES.

Q. Wu[1], Y. Lu[1], J. Shi[3], J. Liu[1, 2] and J. Shi[1]. *[1]Pharmacology, Zunyi Medical College, Zunyi, Guizhou, China, [2]Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS and [3]Pharmacology, Guiyang Traditional Medical College, Guiyang, Guizhou, China.*

Mercury (Hg) and arsenic (As) are frequently found in traditional medicines as sulfides, such as cinnabar (96% as HgS) and realgar (90% as As4S4). The addition of these mineral metals in traditional medicines has been claimed for their therapeutic efficacy. However, Hg is highly toxic, and As is known for its carcinogenic and toxic effects. There is a general perception that any medicinal use of such metal-containing remedies is unacceptable. An opposing opinion is that different chemical forms of arsenic and mercury have different toxic potentials. To clarify this question, cinnabar, realgar, and cinnabar- and realgar-containing traditional medicine An-Gong-Niu-Huang Wan (AGNH), were compared to well-known mercurials (HgS, HgCl2 and MeHg) and arsenicals (As2S2, As2O3, NaAsO2, and Na2HAsO4) for their cytotoxicity in rodent (HAPI, TRL1215) and human (HepG2, Hep3B, FaDu) cell lines. MeHg was most toxic with LC50 of 4-20 μM, followed by NaAsO2 (LC50, 25-250 μM) and HgCl2 (LC50, 50-100 μM), Na2HAsO4 (LC50, 60-400 μM), As2O3 (LC50, 30-900 μM), and As2S2 (LC50, 50-1000 μM). In comparison, the LC50 of realgar ranged from 250 to1500 μM; whereas cinnabar or HgS were approximately 20,000 μM and the toxicity of AGNH was in the range of 1500-8000 μM. Approximately 5,000-fold differences exist between MeHg and HgS, and over 10-fold differences exist between NaAsO2 and As4S4. In conclusion, chemical forms of metals are important factor in determine their toxicity in traditional medicines, both cinnabar and realgar are much less toxic than well-known mercurial and arsenicals.

PS 302 INVESTIGATION OF TELLURIUM TOXICITY IN HT-29 AND CACO-2 HUMAN COLON CELLS.

P. Vij and D. Hardej. *Department of 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 industry for the production of semiconductors, blu-ray discs, as vulcanizing agents and as catalysts for synthetic fiber production suggests that environmental exposure will increase in the future. The neurotoxicity of Te compounds has been documented, particularly in animal studies. Little has been reported regarding the gastrointestinal toxicity of Te, despite the fact that ingestion represents a major exposure route. *In vitro* studies confirming Te toxicity are lacking. The purpose of this study was to investigate the toxicity of tellurium tetrachloride ($TeCl_4$) and diphenylditelluride (DPDT) in Caco2 and Ht-29 human colon cells. Cells from both the cell lines were grown to 90% confluency and treated with concentrations of each compound ranging from 0.98μM to 1.0mM for 24h. Viability was assessed by MTT and bioluminescent-based assays. Significant decreases in viability in each cell line were seen in concentrations ranging from 62.5μM to 1.0mM. Phase contrast and scanning electron microscopy were performed to confirm viability results and to observe morphologic changes consistent with toxicity. Distortion such as disruption of cell structure and blebbing was observed in these groups. In order to investigate the mechanism of cell death, caspase 3/7 and caspase 9 activity was assessed in the HT 29 cells using each compound. Significant increase in caspase 3/7 and caspase 9 activity was observed in 500μM and 1.0mM concentrations indicating cell death by the intrinsic apoptotic pathway. No significant increases in caspases were observed in HT 29 cells treated with $TeCl_4$ at concentrations ranging from 125μM to 1.0mM. We conclude that there is a significant decrease in cell viability in HT 29 and Caco2 cells treated with both compounds in concentrations ranging from 62.5μM to 1.0mM and that HT-29 cells treated with DPDT die via the intrinsic apoptotic pathway.

PS 303 LUNAR DUST SIMULANT IS CYTOTOXIC BUT NOT GENOTOXIC TO HUMAN SKIN FIBROBLAST CELLS.

R. Duffy[1, 2], J. Wise[1, 2], H. Xie[1, 2, 3], A. Jeevaragen[4], W. Wallace[6], D. Hammond[4], T. Shehata[5] and J. P. Wise[1, 2, 3]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME, [2]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME, [3]Department of Applied Medical Science, University of Southern Maine, Portland, ME, [4]NASA Johnson Space Center, Houston, TX, [5]Maine Space Consortium, Augusta, ME and [6]Wyle Integrated Science and Engineering, Houston, TX.*

NASA is considering a permanent space station on the moon. Humans will occupy this space station, therefore it is important to assess any health hazards present in the lunar environment. On prior missions to the moon, the dust that composes the

lunar surface presented potential health problems when it stuck to the astronaut's suits and contaminated the habitation module; NASA is concerned that this dust may be toxic to humans. Lunar dust is composed of silicate minerals that contain various amounts of aluminum and titanium. There are occasionally traces of chromium. Lunar dust simulants were obtained from NASA, in two different particle sizes; fine simulant(JSC-1AF) and very fine simulant (JSC-1AVF). Human skin fibroblast cells (BJhTERT) were used as an in vitro model to investigate the cytotoxicity and genotoxicity of lunar dust and its component particles. We exposed the cells to lunar dust simulants and lunar dust simulant component particle concentrations ranging from 0.1-400 ug/cm2 for an exposure of 24 and 120 h. After 24 and 120 h JSC-1AVF was more cytotoxic than JSC-1AF. Silica and JSC-1AF were similar in cytotoxicity while titanium dioxide displayed a similar cytotoxic effect to that of JSC-1AVF. Zinc chromate was the most cytotoxic of the component particles, followed by chromium oxide, titanium dioxide, with silica being the least cytotoxic. Zinc chromate was found to be moderately genotoxic to BJhTERT cells. Otherwise, our data for 120 h show no chromosome damage induced by lunar dust simulants and lunar dust simulant component particles. This work is supported by the NASA grant EP-08-01, the Maine Space Grant Consortium, and the Maine Center for Toxicology and Environmental Health.

PS 304 COMPARATIVE CYTOTOXICITY OF SIMULATED MOON AND MARS DUSTS, EARTH DUST, AND SELECTED LUNAR DUST COMPONENTS IN HUMAN LUNG EPITHELIAL CELLS.

M. Braun[1, 2], Q. Qin[1, 2], H. Xie[1, 2, 3], R. Leighton[1, 2], M. Mason[2, 4], A. Jeevaragen[5], W. Wallace[5], D. Hammond[5], T. Shehata[6] and J. P. Wise[1, 2, 3]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME, [2]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME, [3]Department of Applied Medical Science, University of Southern Maine, Portland, ME, [4]Department of Chemical and Biological Engineering, University of Maine, Orono, ME, [5]NASA Johnson Space Center, Houston, TX and [6]Maine Space Consortium, Augusta, ME.*

As NASA considers a base on the moon, the potential hazards of lunar dust exposure pose a threat to that colonization. During the Apollo 17 mission, the astronauts reported that the dust was clinging to their suits while they explored the surface. When they returned to the lunar module, dust lifted off their suits and was free floating in their living space. They took off their space suits and dust irritated their skin and lungs. NASA worried that this would hinder future missions and their eventual colonization of the moon. Analysis of the lunar soil at Johnson Space Center in Houston, Texas showed that some of its components were titanium, silica, aluminum, and chromium, the latter of which has already been proven to be a potent carcinogen. We assessed the toxicity of lunar dust simulants and some of its components, and compared it to Earth dust and simulated Mars dust. We used two different lunar dust simulants, JSC-1A-VF (very fine) and JSC-1A-F (fine) and one Mars dust simulant (Mars-1A-F). In addition we considered Earth dust, UPM (urban particulate matter). We exposed immortalized human lung epithelial cells (HBEC-2 clone 1) to particle concentrations of 10-600 ug/cm2 for 24 h. We found that UPM was the most toxic followed by the Mars simulant, AF, and AVF. Furthermore, we concluded that among the lunar dust components we used, chromium was the most toxic. This work is supported by the NASA grant EP-08-01, the Maine Space Grant Consortium, and the Maine Center for Toxicology and Environmental Health.

PS 305 MECHANISMS OF CELLULAR TOXICITY FOR DUAL EXPOSURE TO NICL2 AND COCL2 IN H460 HUMAN LUNG EPITHELIAL CELLS.

C. Lynch and M. Reynolds. *Biology, Washington College, Chestertown, MD.*

Nickel and cobalt, two carcinogens associated with respiratory cancers, can accumulate to toxic levels in the body, producing adverse toxicological effects. We previously found amplified cytotoxic, mutagenic, and genotoxic effects following dual exposure of H460 human lung epithelial cells to these metals compared to single exposure. These effects included increased apoptosis, p53 expression, and micronuclei suggesting a strong connection between dual exposures and the formation of respiratory cancers. The goal of our current study was to elucidate the mechanism of toxicity following dual exposure to NiCl2 and CoCl2, specifically examining the roles of reactive oxygen species (ROS), p53, and caspase activity. Western blot analysis revealed a dose-dependent increase in double strand breaks as measured by the phosphorylation of H2AX. In order to determine if the formation of double strand breaks was the result of reactive oxygen species, cells were pretreated with N-acetyl cysteine, an antioxidant. This resulted in decreased H2AX, p53, and caspase 3 activity. Colony formation and western blot analysis with siRNAp53 cells suggested cytotoxicity and genotoxicity was p53-independent. However, mutagenicity as measured by micronuclei formation was found to be p53-dependent. Finally, western blot analysis and caspase 3/7 luminescence assays revealed that caspase activity is increased following dual exposures, and supports a caspase-dependent mechanism of cytotoxicity. Overall, these results indicated that dual exposure to nickel and cobalt result in increased cytotoxicity, mutagenicity, and genotoxicity, which are likely the result of ROS production and caspase activation.

PS 306 RAD23, A FACTOR INVOLVED IN UBIQUITIN-PROTEASOME SYSTEM, REDUCES METHYLMERCURY TOXICITY THROUGH INHIBITION OF DEGRADATION OF N-GLYCANASE IN BUDDING YEAST.

G. Hwang and A. Naganuma. *Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi, Japan.*

We have previously reported that the ubiquitin-proteasome system (UP system) plays an important role in the protection against methymercury (MeHg) toxicity in budding yeast. We also found that overexpression of Rad23 confers resistance to MeHg in budding yeast through increase of stability of certain proteins that was degraded by the UP system. In this study, we screened the as-yet-unidentified protein(s) that was involved in reduction mechanism of methylmercury toxicity by Rad23. We examined effects of overexpression of nine proteins that were known to bind to Rad23 on the sensitivities of yeast cells to MeHg. The yeast cells that overexpressed Ddi1, Png1 or Ufd2 showed strong resistance to MeHg, as compared with the control cells. Among these proteins, we performed further detailed examination about Png1. The levels of Png1 protein (Png1p) were remarkably increased by overexpression of Rad23 or treatment with a proteasome inhibitor. Moreover, after treatment of yeast cells with cycloheximide, the levels of Png1p were decreased time dependently but the decrease of Png1p levels were significantly inhibited by overexpression of Rad23. However, degree of the resistance to MeHg induced by overexpression of Rad23 was smaller in Png1-defective yeast compared with that of the wild-type strain. These results suggested that Png1 was partly necessary for the resistance to MeHg induced by overexpression of Rad23. Thus, it is possible to conclude that the levels of Png1p were usually controlled by the UP system, but the levels were increased in Rad23-overexpressed cells and then the cells acquired resistance to MeHg. Png1p has a role as N-glycanase in cells and N-glycanase is involved in the control system of quality of endoplasmic reticulum. Interestingly, we found that the function of Png1 as a N-glycanase is essential for resistance to MeHg suggesting that N-glycanase might play an important role in the protection against MeHg toxicity.

PS 307 METHYLMERCURY INDUCED PANCREATIC β-CELL APOPTOSIS THROUGH ENDOPLASMIC RETICULUM STRESS.

T. Lu[1], D. Hung[1, 2], C. Chen[1, 3], C. Yen[4], C. Su[5], Y. Chen[6] and C. Huang[7]. *[1]Graduate Institute of Drug Safety, China Medical University, Taichung, Taiwan, [2]Toxicology Center, China Medical University Hospital, Taichung, Taiwan, [3]Department of Emergency, China Medical University Hospital, Taichung, Taiwan, [4]Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, Taiwan, [5]Department of Otorhinolaryngology, Head and Neck Surgery, Changhua Christian Hospital, Changhua, Taiwan, [6]Department of Physiology, China Medical University, Taichung, Taiwan and [7]School of Chinese Medicine, China Medical University, Taichung, Taiwan.*

Mercury is a well-known highly toxic metal and can induce oxidative stress damage causing cell death. Pancreatic β-cells are vulnerable to oxidative stress. However, the toxic effects of MeHg-induced pancreatic β-cells apoptosis remain unclear. The present study was designed to investigate the cytotoxicity and its possible mechanisms of MeHg in rat pancreatic β-cells (RIN-m5f cells). Here, we found that MeHg dose-dependently decreased cell viability and significantly induced ROS formation and caspase-3 activity in RIN-m5f cells. MeHg also displayed several features of mitochondrial-dependent apoptotic signals, including disruption disruption of the mitochondrial membrane potential, increase of cytochrome c release, upregulations of Bax, Bak, and p53 gene expression, and activations of caspase cascades. Moreover, treatment of RIN-m5f cells with MeHg also resulted in trigger endoplasmic reticulum (ER) stress, as indicated by increase the expression and activities of glucose-regulated protein 78 (GRP-78), C/EBP homologous protein (CHOP), calpain-I/-II and caspase-12. Meanwhile, the increases of mitogen-activated protein kinase (MAPK) activations were shown after treatment of RIN-m5f

cells with MeHg. N-acetylcysteine effectively reversed the MeHg-induced cell apoptosis-related signals. Taken together, our data suggest that MeHg-induced oxidative stress causes pancreatic β-cells apoptosis, and both mitochondrial-dependent and ER stress- mediated apoptotic signals involve in the MeHg-induced pancreatic β-cells death.

PS 308 ROLES OF RIP1 AND YIL006W, MITOCHONDRIAL PROTEINS, IN METHYLMERCURY TOXICITY IN BUDDING YEAST.

J. Lee, G. Hwang and A. Naganuma. *Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan.*

Methylmercury (MeHg) is an environmental pollutant that causes severe damage in central nervous system. However, the molecular mechanism of MeHg toxicity is not fully understood. Several investigators have demonstrated that mitochondria are potentially involved in MeHg toxicity. In order to characterize functional relationship between MeHg toxicity and mitochondria, we searched for mitochondrial proteins involved in MeHg toxicity in budding yeast. Yeast is a genetically well-characterized eukaryotic organism that shares many genes common with mammals, including humans. We recently found that yeast cells deficient in Yil006w exhibited resistance to MeHg as compared to wild-type yeast. Yil006w is one of the transporter families existing in inner-membrane of mitochondria. Overexperssion of Yil006w conferred hypersensitivity to MeHg. Moreover, not only cellular protein level, but also mRNA level of Yil006w was increased by MeHg treatment. Mitochondrial level of Yil006w was also increased by treatment of MeHg. These results suggested that MeHg might increase the mitochondrial Yil006w level and strengthen its toxicity. We previously demonstrated that Rip1, a component of mitochondrial electron transfer chain complex III, was potentially involved in the production of reactive oxygen species (ROS) in response to MeHg. Therefore, we examined relationship between Rip1 and Yil006w in MeHg toxicity. We treated yeast cells, which lacked both Rip1 and Yil006w, with MeHg. The degree of resistance to MeHg was not increased by the deletion of both Rip1 and Yil006w as compared to each single-deletion mutants, suggesting that Rip1 and Yil006w strengthen MeHg toxicity through a mechanism involved in a common pathway. We also found that the increase of ROS level induced by MeHg was significantly attenuated in Yil006w-deficient yeast. Thus, Yil006w might have a major role in the mechanism of Rip1-enhanced ROS generation in response to MeHg.

PS 309 MERCURY MODULATE THE CYTOCHROME P450 1A1 IN C57BL/6 MICE: *IN VIVO* AND *IN VITRO* STUDIES.

I. Amara and A. El-kadi. *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.*

Heavy metals, such as mercury (Hg2+), and aryl hydrocarbon receptor (AhR) ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are environmental co-contaminants and their molecular interaction may disrupt the coordinated regulation of the carcinogen-activating enzyme cytochrome P4501a1 (Cyp1a1). Therefore, in the current study we examined the effect of Hg2+ on the expression of Cyp1a1 in C57BL/6 mice livers and isolated mouse hepatocytes. For this purpose, C57BL/6 mice were injected intraperitoneally with Hg2+ (2.5 mg/kg) in the absence and presence of TCDD (15 μM/kg). After 6 and 24 h the tissue was harvested and the expression of Cyp1a1 mRNA, protein and catalytic activity levels were determined using real time-PCR, Western blot and 7-ethoxyresorufin O-deethylase (EROD) analyses, respectively. In vitro, isolated mouse hepatocytes were incubated with TCDD (1 nM) in the presence and absence of various concentrations of Hg2+ (2.5, 5 and 10 μM). Our results demonstrate that at in vivo level, Hg2+ significantly decreased the TCDD-mediated induction of Cyp1a1 mRNA at 6 h while potentiating its mRNA, protein, and catalytic activity levels at 24 h. At in vitro level, Hg2+ significantly inhibited the TCDD-mediated induction of Cyp1a1 at mRNA, protein and activity levels in a concentration-dependent manner. Investigating the effect of Hg2+ at transcriptional levels revealed that Hg2+ significantly inhibited the TCDD-mediated induction of AhR-dependent luciferase reporter gene expression. In conclusion, caution should be taken when extrapolating in vitro data to in vivo situation. Furthermore, our results indicate more complex regulation of Cyp1a1 at the in vivo level that warrants further investigation. Funded by NSERC Discovery Grant RGPIN 250139-07.

PS 310 ASSESSING METAL LEVELS IN CHILDREN FROM THE MECHANISTIC INDICATORS OF CHILDHOOD ASTHMA (MICA) STUDY.

A. Sanders[1], J. Gallagher[2], J. McGee[2], S. Rhoney[2], E. Hugens[2], H. Özkaynak[3] and R. Fry[1]. [1]*Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC,* [2]*National Health Environmental Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC and* [3]*National Exposure Research Laboratory, U.S. EPA, Research Triangle Park, NC.*

Toxic and essential metals levels can be used as health indicators. Here we quantitatively compare and contrast toxic and essential metals levels in vacuum dust, urine, and fingernail samples of 109 children in Detroit, Michigan as part of The Mechanistic Indicators of Childhood Asthma (MICA) study – a study aimed at examining relationships between exposure and biomarkers of exposure effects and susceptibility in children ages 9-13. Comparison of the Detroit children's urinary levels with the CDC's Fourth National Report on Human Exposure to Environmental Chemicals showed that urinary metal levels for As, Cd and Pb, were similar to nationally reported levels. Creatinine-adjusted urinary metal concentrations were in the order: Zn >> Se > Cu > As > Ni > Pb > Cd > Cr > V > Mn. We find no statistical differences in essential metals levels between children with varied vitamin use status. Our results indicate that levels of toxic metals indeed covary with each other as well as with essential metals (e.g. arsenic and selenium) in the biological matrices. Furthermore, among all possible pairwise comparisons of metals and media types, metals levels in the matrices representing chronic exposure (nails and dust) were more closely associated relative to the short term exposure indicator (urine). This study underscores the importance for the consideration of both individual and multiple metals in health effect studies. This abstract is the text of a proposed presentation and does not represent EPA policy.

PS 311 BRIDGING THE MISSING LINK IN PRINCIPLE THEORY – SELENIUM TRANSPORT IN ENTEROCYTES AND HEPATOCYTES.

S. Misra[1], R. W.M. Kwong[2] and S. Niyogi[1]. [1]*Biology, University of Saskatchewan, Saskatoon, Saskatchwean, Canada and* [2]*Toxicology Centre, University of Saskatchewan, Saskatoon, Saskatchwean, Canada.*

Selenium (Se) is a unique trace element with diverse physiological functionality. In contrast, it exhibits very low margin between essentiality and toxicity. Recent research reveals potential applications of Se in cancer prevention and treatment. Nevertheless, a simple, yet basic research question remains to be understood in depth – how Se is transported in higher eukaryotic cells? To address this, the present study was designed to evaluate Se transport kinetics at physiological concentrations using isolated rainbow trout hepatocytes and enterocytes as model systems. Selenite transport was linear in both cell types at the concentration range tested. However, pre-incubation of selenite with glutathione (GSH) resulted in increased Se uptake with saturable uptake kinetics in enterocytes but not in hepatocytes during early uptake phase. L-cysteine, another thiol-group containing agent, increased Se uptake in both cell types by ~10-folds when pre-incubated with selenite. Inhibitory effects of anionic transporter blocker, DIDS and analogous chemical compounds (sulfite) suggested specific carrier mediated uptake of both forms. However, DCCD and orthovanadate couldn't block Se uptake, indicating an energy-independent process. We then tested the hypothesis whether mercury can block both forms of Se uptake or not. Mercury exerted uptake inhibition in both cell types except reduced form (selenite + GSH) of Se in enterocytes. These observations indicate the possibility of at least two different pathways of inorganic Se uptake in enterocytes. However, when both cell types were pre-exposed in combination with mercury and DIDS, the inhibitory effect was higher than DIDS but was comparable with mercury alone. Absence of any synergistic effects in combining exposure raises the possibility of inhibition of anionic transporter by mercury. Overall, our data suggests a passive carrier mediated uptake of inorganic Se in both cell types.

PS 312 THE IRON TRANSPORTER FERROPORTIN CAN ALSO FUNCTION AS A MANGANESE EXPORTER.

M. S. Madejczyk and N. Ballatori. *Environmental Medicine, University of Rochester School of Medicine, Rochester, NY.*

Manganese (Mn) is an essential trace element that is utilized in a number of important cellular processes; however, excess Mn can also be quite toxic. Chronic exposure to high levels of Mn leads to neurological damage with psychiatric symptoms as well as disruptions in motor function. The mechanisms by which Mn is transported across cell membranes to reach its target sites are now beginning to be elucidated, although much remains to be learned about these processes, and in particu-

lar about cellular export mechanisms. The present study examined the hypothesis that the iron exporter ferroportin (FPN1) mediates cellular Mn export, using *Xenopus laevis* oocytes expressing human FPN1. When compared to oocytes expressing only the divalent metal transporter-1 (DMT1), ^{54}Mn accumulation was lower in oocytes also expressing FPN1. FPN1 expressing oocytes were able to significantly export ^{54}Mn when compared to control oocytes when injected with 16 μM Mn (27% vs 7.5% respectively, over 4 h at pH 7.4). Export was concentration-dependent and could be partially *cis*-inhibited by 100 μM Fe, Co, and Ni, but not Rb. In addition, transport ability was lost at pH 5.5, but not at pH 8.5. Incubation in high K media was also able to partially inhibit Mn efflux in FPN1 expressing oocytes. These results indicate that Mn is a substrate for FPN1, and the driving force is both pH and membrane potential-sensitive.

PS 313 CHARACTERIZATION OF ZIP8 AND ZIP14 ZINC/BICARBONATE SYMPORTERS IN XENOPUS OOCYTES: CADMIUM AND ZINC UPTAKE AND INHIBITION BY VARIOUS METALS.

M. Gálvez-Peralta, E. B. Hay, H. Li, M. Soleimani, E. Johansson, L. He, B. Wang and D. W. Nebert. *University Cincinnati Medical Center, Cincinnati, OH.*

Cadmium (**Cd**) is a non-essential metal. Because of increases in cigarette smoking and the industrialization growth, Cd exposure in our environment is increasing dramatically. Chronic exposure to low doses of Cd can cause renal disease and osteomalacia. Cd is classified by IARC as a Category I human lung carcinogen. Cd uptake by mammalian cells uses Ca^{2+}channels and SLC11A2 (**DMT1**). Previous studies from this lab have shown that bicarbonate-dependent Cd uptake is facilitated by SLC39A8 (**ZIP8**) and SLC39A14 (**ZIP14**) in mammalian cells. ZIP14 and ZIP8 are divalent cation/$HCO3^-$ symporters, whose endogenous function is primarily zinc uptake. **ZIP14A** and **ZIP14B** are distinctly different splice variants of exon 4. In addition to Cd—Hg, Pb, Pt and U are known to cause human renal Fanconi syndrome (renal proximal tubular acidosis). In this work, we inserted mouse ZIP8, ZIP14A and ZIP14B cDNA coding regions into the Xenopus vector pXFRM and performed transcription; capped RNA (**cRNA**) was microinjected into Xenopus oocytes. Km and Vmax values for Zn and Cd uptake were determined; competitive inhibition of Zn and Cd uptake by the three ZIP symporters was studied for Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Pt^{2+}, U^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Cs^{1+}, Mn^{2+} and Fe^{2+}. We found that Pt, U, Mn, Fe, Ni and Cs are significant inhibitors of Cd uptake by ZIP8; only high concentrations of Zn, Pt, Co, Mn and Cs inhibited Cd uptake by ZIP14A and ZIP14B. Differences in response were also observed for Zn uptake by the three ZIPs. Little is known about how ZIP14A and ZIP14B organ-specific expression is regulated and why the two forms persist evolutionarily, but this study shows they differ in Cd and Zn uptake and competition against different metals. This study also suggests strongly that ZIP8 might play a major role in metal-induced renal Fanconi syndrome.-*Supported by NIH grants R01 ES010416, P30 ES06096 (D.W.N.), T32 ES016646 (M.G.-P.), & R01 DK062809 (M.S.)*

PS 314 ZIP8 AND ZIP14 ZINC/BICARBONATE SYMPORTERS: THEIR POSSIBLE ROLES DURING INFLAMMATION.

Z. Wang[1], M. Gálvez-Peralta[1], C. M. Williams[1], M. Liu[2], D. L. Knoell[2] and D. W. Nebert[1]. [1]*Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH and* [2]*David Heart and Lung Research Institute, Ohio State University, Columbus, OH.*

SLC39A8 (**ZIP8**) and SLC39A14 (**ZIP14**) are divalent cation/$HCO3^-$ symporters, primarily used for Zn uptake in vertebrate cells. Cd and other nonessential metals enter vertebrate cells by displacing Zn. SLC39A14 has two exons 4, giving cell-type-specific expression of **ZIP14A** and **ZIP14B** as alternatively-spliced products, whereas SLC39A8 encodes a single product. Because Zn is involved in the host response to inflammatory stress, and inflammation induces hypozincemia, the objective of this project is to determine whether different inflammatory agents are able to induce changes in the expression of these transporters, and if this is organ-specific.

C57BL/6J male mice were treated i.p. with TNF (5 μg/kg), IL6 (100 μg/kg) or LPS (5 mg/kg) and 12 tissues were collected at different times for qRT-PCR and Western blot. Because metallothionein and SLC11A2 (**DMT1**) have been associated with inflammation, their mRNA levels were also measured. To correlate changes in ZIP8, ZIP14A and ZIP14B with inflammation, we also measured TNF, IL6, and IL1B mRNA levels. We found significant differences in the response of each organ, and ZIP mRNAs were induced at different times and patterns: heart showed the most highly TNF-induced ZIP8, ZIP14B mRNA levels; whereas ZIP8 and ZIP14A mRNA reached their highest levels at 8 h, the peak of ZIP14B expression occurred when the levels of ZIP8 and ZIP14A were decreasing (16 h). There was a double peak for interleukins in liver, heart and brain (1st peak at 2-4 h; 2nd peak at 16 h), suggesting an early vs late response; this pattern was also seen for ZIP14B mRNA. ZIP8 and ZIP14A mRNA levels were unchanged in liver or testis. Kidney ZIP8 and ZIP14A mRNA levels were significantly diminished. These observations will help us understand how hypozincemia is regulated in various tissues, following an inflammatory response.-Supported, in part, by NIH grants R01 ES010416 and P30 ES06096 (D.W.N.), T32 ES016646 (M.G.-P.) & R01 HL08698 (D.L.K.)

PS 315 EVALUATION OF MUTAGENICITY, CHROMOSOME BREAKAGE, AND DNA DAMAGE BY PIG-A, MICRONUCLEUS AND COMET ASSAYS IN 28-DAY REPEATED DOSE TOXICITY STUDIES.

J. Shi[1], M. Paranjpe[1], S. W. Bruce[1], T. Kelley[1], S. Springer[1], J. Sly[1], M. Klug[1], M. Arevaro[1], S. Atta-Safoh[1], F. Debelie[1], L. Krsmanovic[1], P. Sareen[1] and S. Dertinger[2]. [1]*BioReliance Corporation, Rockville, MD and* [2]*Litron Laboratories, Rochester, NY.*

Integration of multiple in vivo genotoxicity endpoints into repeat dose general toxicity studies not only allows evaluating genotoxic potential of the test compounds with several endpoints which detect different genotoxic mode of action (MOA) in different organs, but also has the potential to reduce animal use. Here, we evaluated two genotoxic compounds, 7,12-dimethylbenz(a)anthracene (DMBA) and diethylnitrosamine (DEN) for Pig-a gene mutation and chromosomal damage in peripheral blood, and primary DNA damage in liver and blood cells in Sprague Dawley rats treated daily for 28 days. An additional treatment was performed 3 hours prior to necropsy on Study Day 29 for DNA damage evaluation. Peripheral blood samples obtained on Study Days -1, 15 and 29 were analyzed by flow cytometry to determine the frequencies of CD59-negative erythrocytes and reticulocytes (indicative of Pig-a gene mutation). Frequencies of micronucleated reticulocytes (%MN-RETs) were determined in peripheral blood obtained on Study Days 4 and 29 by flow cytometry. As a result, DMBA induced dose-dependent increases of CD59-negative erythrocytes/reticulocytes and MN-RETs, but no significant increase in DNA damage in liver cells when tested up to 10 mg/kg/day, which appears to be below the maximum tolerated dose. When tested up to 200 mg/kg/day in a follow up acute study, DMBA was determined positive in the liver Comet assay. On the other hand, DEN produced negative results in both the Pig-a mutation assay and the MN assay, and evaluation of Comet slides are still in progress. The different responses observed in the three genotoxicity endpoints induced by DMBA or DEN provided a characterization of the genotoxic MOA, target organs and a comparison of assay sensitivity under the experimental conditions.

PS 316 A NOVEL HIGH-THROUGHPUT *IN VITRO* MNT PROFILING ASSAY FOR SUSPENSION CELLS.

S. Kirchner, M. Prummer, S. Marget-Müller, J. Muller, C. McGinnis, K. Kolaja, R. Werder, K. Christensen, S. Zoffmann, T. Enderle, R. Rietmann, D. Vögelin, P. Iaiza, C. Fattinger and T. Weiser. *F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland.*

The in vitro micronucleus assay is well established as an early tool to evaluate the aneugenic and/or clastogenic activity of potentially new drug candidates. The recent acceptance of OECD TG487 by regulatory agencies positions the in vitro MNT as an alternative for both the GLP in vitro chromosomal aberration assay and the L5178Y Mouse Lymphoma assay. This allows for the first time to use the same assay and cell system throughout the different stages of testing in the drug development process with its specific needs. So far standard suspension cell lines used for in vitro micronucleus evaluation such as L5178Y and TK6 were only accessible to true high throughput applications by means of flow cytometry not allowing for analysis on a single cell level. We are presenting here a novel and universal approach to prepare arrays of non-adherent cells from 96-well plates for automated microscopy and micronucleus evaluation to fill this gap. For image segmentation and object classification we co-developed an adaptive and iterative algorithm for L5178Y cells using Definiens Cognition Network Technology and non-parametric statistical methods were applied to classify the micronucleation potential. To validate the assay protocol we compared the results obtained between the high throughput 96-well plate method and a regulatory like in vitro micronucleus screening assay for selected reference and 224 in house compounds using L5178Y cells. Based on these experiences, this high throughput in vitro MNT approach will be compared and contrasted within the context of supporting the early development of drug candidates. In summary, these developments open up new possibilities for aligning the clastogenicity/aneugenicity assessment throughout the drug development process from HT approaches to regulatory like screening and regulatory testing when using suspension cells. In addition this development might also have the potential to make human primary lymphocytes more accessible to higher throughput applications.

PS 317 THE HUNT FOR MISLEADING POSITIVES: CAN NON-GENOTOXIC REGULATION OF GADD45A INTERFERE WITH GREENSCREEN HC?

C. Topham[2] and R. Walmsley[1, 2]. [1]*Life Sciences, University of Manchester, Manchester, United Kingdom and* [2]*Gentronix Ltd., Manchester, United Kingdom.*

The GADD45a-GFP 'GreenScreen HC' genotoxicity assay (GSHC) has high specificity (95%) and sensitivity (87%) in the prediction genotoxic carcinogenicity. Accordingly, the frequency of misleading positive GSHC results is low when compared with other mammalian in vitro genotoxicity assays. The GSHC assay employs the response of the DNA damage-inducible gene GADD45a as a marker of genotoxic stress, using a GADD45a-GFP reporter containing key regulatory elements.
In order to rigorously assess the specificity of the GSHC assay in light of the current knowledge of the regulation of GADD45a, chemicals with the potential to induce a GADD45a response in the absence of genotoxic stress were sought in order to expose the assay to challenging new biological mechanisms. Examples of such chemicals included activators of signalling pathways known either to positively regulate GADD45a, such as p53. or inhibitor negative regulators of GADD45a, such as NF-κB and Bcl-2 family members. A third group included chemicals known to activate apoptosis by various cytotoxic stress signalling pathways. Stimulation of an apoptotic response by these chemicals was assessed by monitoring of levels of caspase 3/7 activation, annexin V binding and DNA fragmentation via TUNEL.
Six proprietary and 17 non-proprietary compounds were assessed with GSHC. Published comparative genotoxicity data for these chemicals were either sparse or non-existent, so genotoxicity data were generated in this study using the in vitro micronucleus test (MNT) and the comet assay, as well as in silico alerts from the DEREK for Windows SAR software. Chemicals with positive GSHC data were also positive in either the MNT or Comet assay. These data confirm validation studies which demonstrate that compounds producing a unique positive response in the GADD45α-GFP assay are rare, even amongst a group of compounds inducing apoptosis through non-genotoxic mechanisms, which might be considered high risk for the generation of 'false' positives in this assay.

PS 318 COMPARATIVE DNA DAMAGE AND CHRONIC LIVER TOXICITY OF BENZO[A]PYRENE IN TWO POPULATIONS OF THE ATLANTIC KILLIFISH (*FUNDULUS HETEROCLITUS*) WITH DIFFERENT EXPOSURE HISTORIES.

R. Di Giulio[1], D. Jung[2, 1] and L. Wills[3, 1]. [1]*Nicholas School of the Environment, Duke University, Durham, NC,* [2]*Department of Physiology, Dartmouth Medical School, Lebanon, NH and* [3]*Medical University of South Carolina, Charleston, SC.*

The Atlantic Wood Industries Superfund site on the Elizabeth River (ER) in Portsmouth, VA is contaminated with polycyclic aromatic hydrocarbons (PAH) derived from creosote. Embryos and larvae of ER killifish (Fundulus heteroclitus) are refractory to the induction of enzymes regulated by the aryl hydrocarbon receptor (AHR) including cytochrome P4501A (CYP1A) and are resistant to PAH-induced lethality and teratogenicity. However, adult ER killifish collected from this site show a greater prevalence of hepatic and pancreatic tumors compared to those from reference sites. This study examined the relative sensitivities of laboratory-reared offspring from the two populations to DNA damage and chronic liver exposure following exposures to benzo[a]pyrene (BaP). Larvae from the ER and a reference site on King's Creek (KC) were subjected to two 24 hr aqueous exposures to BaP (0-400 μg/L). At various time points, larvae were analyzed for CYP1A activity, BaP concentrations, nuclear and mitochondrial DNA damage, and liver pathology. CYP1A was induced by BaP in KC but not ER larvae, and KC larvae demonstrated a greater reduction in whole body concentrations of BaP over time. Mitochondrial and nuclear DNA lesion frequency increased significantly in BaP exposed KC larvae, but not in those from the ER. Nine months post-exposure, KC juveniles exhibited significantly more hepatic foci of cellular alteration (FCA), and only KC juveniles developed hepatocellular carcinomas. Thus, in addition to acquiring the heritable resistance to the acute teratogenic effects of PAHs, ER fish appear to have concomitantly developed resistance to chronic effects, including cancer.

PS 319 DNA DAMAGE CAUSED BY TRICYCLIC ANTIDEPRESSANTS.

E. A. Korobkova and A. K. Williams. *Science, John Jay College of Criminal Justice, New York, NY.* Sponsor: B. Shane.

Tricyclic antidepressants were discovered in the 1950s and were used for many years in the treatment of mood disorders. The antidepressants and their metabolites can be very genotoxic in living cells. The planar structures of the drugs can insert between DNA bases forming stacking complexes. The metabolism of antidepressants may lead to the DNA bases modifications or DNA strand breaks. We studied the effect on DNA of three tricyclic anatidepressants, imipramine, amitriptyline, and opipramol. We focused on the drug-DNA binding and DNA damage aided by peroxidase catalysis. As a model of peroxidase we used HRP (Horseradish peroxidase). We performed ethidium bromide fluorescence quenching experiments and determined drug concentrations at 50% fluorescence quenching, C50. The value of C50 ranged from 1 mM for opipramol to 5 mM for imipramine and amitriptyline. Agarose gel electrophoresis studies showed that DNA disappears in the reaction mixtures containing imipramine and HRP/H2O2. Phenol:chlorophorm:iso-amyl alcohol extraction from the mixtures containing DNA and imipramine in the presence of HRP/H2O2 indicated that DNA degrades in the reaction. UV-Vis studies showed that both imipramine and opipramol are the substrates for HRP. At pH 7, reaction between HRP and excess of H2O2 and imipramine led to the formation of a broad spectrum with a peak at 522 nm. The intensity of the spectrum increased with time. The position of the maximum shifted to the longer wavelengths as the pH decreased reaching 650 nm at pH 2. These spectra are associates with imipramine radical. GC-MS analysis of the brown precipitate produced in the mixture of imiprmaine and HRP/H2O2 indicated the dealkylation process and the formation of iminodibenzyl. Thus all three antidepressants bind DNA possibly by intercalation, opipramol exhibiting a greater affinity compared to imipramine and amitriptyline. DNA degrades in the presence of imipramine and HRP/H2O2 at the drug concentration of 2 μM. The damage to DNA is caused by imipramine reactive intermediate.

PS 320 EVALUATION OF DIRECT AND INDIRECT DNA DAMAGE INDUCED BY OCHRATOXIN A IN CHO AND TK6 CELLS USING THE COMET ASSAY.

R. Ali[1, 2], J. G. Shaddock[2], W. Ding[2], J. A. Bhalli[2], Q. M. Khan[1] and R. H. Heflich[2]. [1]*Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan and* [2]*Division of Genetic and Molecular Toxicology, National Center for Toxicological Research /U.S. FDA, Jefferson, AR.* Sponsor: M. Moore.

The fungal toxin, Ochratoxin A (OTA), a common contaminant in human food and animal feed, has been reported to be mutagenic, clastogenic, and aneugenic in mammalian cells. The present study assessed the direct and indirect (oxidative) DNA damage induced by OTA in two mammalian cell lines, CHO-K1-BH4 Chinese hamster ovary cells and TK6 human lymphoblastoid cells. DNA damage was evaluated using the Comet assay, with and without digestion by formamidopyrimidine-DNA glycosylase (fpg) to cleave at oxidized purine adducts. The cells were treated with 5, 10, 20, 30, 40 and 50 μM OTA for 4 hr; the treatments resulted in >70% viability, as estimated by Trypan Blue dye exclusion. The 4 hr exposure to OTA did not induce direct DNA damage [as measured by Tail Intensity % (TI%)] in CHO cells, but it did induce a significant dose-responsive increase in direct DNA damage in TK6 cells. In addition, OTA exposure resulted in a dose-responsive induction of fpg-sensitive sites in both cell lines, indicative of the induction of oxidative DNA damage. Significant induction of fpg-sensitive sites was detected at OTA concentrations of >20 μM for CHO cells and >30 μM for TK6 cells. The results of this study suggest that oxidative DNA damage is involved in the genotoxicity produced by OTA in mammalian cells.

PS 321 THE *IN VIVO* RAT COMET ASSAY OF DIETHYLNITROSAMINE: RELEVANCE BETWEEN CARCINOGENESIS AND DNA DAMAGE IN THE TARGET ORGANS.

Y. Uematsu, C. Hirogaki, H. Adachi, K. Kijima, T. Yamada, Y. Michimae, I. Matsumoto, T. Koujitani, H. Oishi, H. Funabashi and T. Seki. *Safety Research Laboratory, Dainippon Sumitomo Pharmacology Co., Ltd., Suita-city, Osaka, Japan.*

Diethylnitrosamine (DEN) is a DNA alkylating N-nitroso compound which induces tumors in the rat liver, esophagus and kidney. Especially in the kidney, tumors were reported to be induced specifically in the cortical epithelium. In this study, to understand the relationship between carcinogenesis and the induction of DNA damage, we investigated the DNA damage of the target organs using the single cell gel electrophoresis assay (Comet assay). DEN was administered orally once a day 3 times to male 8 week-old SD rats. Based on the results of the preliminary study, we set 200 mg/kg as the highest dose group. In the Comet assay, rats were euthanized 3 hours after the last dosing. The liver, kidney cortex, kidney medulla, grandular stomach and small intestine were obtained and isolated cells of each organ were analyzed in the Comet assay. The % tail DNA of comet images was analyzed for 100 nuclei per organ. A small portion of each organ was fixed in 10% phosphate buffered formalin, embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin and subjected to histopathological examination. The % tail DNA in the liver and kidney cortex peaked in 50 mg/kg/day group and decreased gradually in higher dose groups. On the other hand, the % tail

DNA in the kidney medulla, grandular stomach and small intestine did not significantly increased up to 200 mg/kg/day group. No cytotoxic observation was found in any organ at 50 mg/kg/day group except slight centrilobular single cell necrosis was found in the liver. These findings indicate that the Comet assay detected DNA damage in the tumor target sites. In the kidney, these results suggest that DEN exert genotoxic effects specifically in the kidney cortex.

PS 322 OPTIMAL CONDITIONS FOR PERFORMANCE OF THE COMET ASSAY USING A THREE-DIMENSIONAL HUMAN EPIDERMAL MODE.

H. Kojima and M. Hojyo. *Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan.*

To evaluate the risk of genotoxicity when human skin is exposed to chemicals, we attempted to determine optimal conditions for the comet assay using a three-dimensional human epidermal model (LabCyte EPI-MODEL: epidermal model, Japan).
In this study, the well-characterized metabolically activated genotoxicants, cyclophosphamide (CP), 2-aminoanthracene (2-AA), and nitrosodimethilamine (NDA), were utilized for the comet assay using an epidermal model to evaluate metabolic activation of chemicals.
The comets of 2-AA and NDA increased dose-dependently on the epidermal models treated for 24 hours, while the results were ambiguous when treatment lasted for four hours. CP, on the other hand, did not induce comets.
We consider the comet assay using an epidermal model to be potentially useful in being able to detect indirect genotoxicants regardless of metabolic activation.

PS 323 GENOTOXICITY OF STYRENE-ACRYLONITRILE TRIMER IN WEANLING F344 RATS.

R. S. Chhabra[1], C. A. Hobbs[2], L. Recio[2], J. Winters[2], K. Shepard[2], P. Allen[2], M. Prajapati[2], M. Streicker[3] and K. L. Witt[1]. [1]*National Toxicology Program, NIEHS, Research Triangle Park, NC,* [2]*Genetic and Molecular Toxicology, ILS, Inc., Research Triangle Park, NC and* [3]*Investigative Toxicology, ILS, Inc., Research Triangle Park, NC.*

Styrene-acrylonitrile trimer (SAN trimer) is a byproduct in production of acrylonitrile styrene plastics. The chemical was identified at a Superfund site in Dover Township, NJ and was tested by the National Toxicology Program (NTP) in a 2-yr perinatal dosed-feed study in rats. It was also tested by the NTP in an Ames assay and was negative. To further characterize the toxicity of SAN trimer, the NTP evaluated its genotoxicity in a combined micronucleus (MN)/Comet assay in 4-wk old male and female F344 rats. SAN trimer was administered by gavage (37.5, 75, 150, or 300 mg/kg) once daily for 4 days. The lower doses approximated those used in the NTP 2-year feeding study. Samples were collected 4 hr after final dosing for measurement of micronucleated reticulocyte (MN-RET) frequencies in blood by flow cytometry and DNA damage in blood, liver, and brain (cerebrum and cerebellum) using the Comet assay. A dose-related increase ($p < 0.0001$) in MN-RET was seen in both male and female rats administered SAN trimer; significant increases in MN-RET were seen in the 300 mg/kg males and the 150 and 300 mg/kg females. The RET population was reduced in the high dose male rats, suggesting chemical-related bone marrow toxicity. Dose-related increases in DNA damage, measured as % tail DNA in the Comet assay, were seen in the brains of both male ($p < 0.0074$) and female ($p < 0.0001$) rats; significantly increased damage was seen in the 300 mg/kg males and the 150 and 300 mg/kg females compared to controls. Increased levels of DNA damage were also seen in liver cells and leukocytes of male and female rats treated with SAN trimer. No indication of chemical-related cytotoxicity was seen in any of the tissues examined for DNA damage. In summary, the results of this subacute MN/Comet assay indicate induction of significant genetic damage in multiple tissues of weanling F344 male and female rats after oral exposure to SAN trimer.

PS 324 THE EVALUATION OF MN AND SCE FREQUENCIES AND OXIDATIVE STRESS STATUS OF HOSPITAL STAFF OCCUPATIONALLY EXPOSED TO LOW DOSES OF IONIZING RADIATION. DOES THE LOW DOSE STIMULATE DEFENSE STATUS?

A. Aydin[1], A. Eken[2], O. Erdem[2], C. Akay[2], B. Soykut[2], A. Sayal[2] and I. Somuncu[3]. [1]*Toxicology, Yeditepe University, Faculty of Pharmacy, Istanbul, Turkey,* [2]*Toxicology, Gulhane Military Medical Academy, Ankara, Turkey and* [3]*Radiology, Gulhane Military Medical Academy, Ankara, Turkey.* Sponsor: A. Karakaya.

Ionizing radiation is known to induce oxidative stress through generation of reactive oxygen species (ROS) resulting in imbalance of the pro-oxidant and antioxidant activities and cellular DNA damage. The aim of this study was to evaluate the oxidative stress status and the genotoxic effects in radiology unit staff. Antioxidant enzyme activities and malondialdehyde (MDA) levels were measured. The micronucleus (MN) and sister chromatid exchanges (SCE) techniques were used. This study included 40 exposed individuals together with 30 control subjects. The activities of erythrocyte CuZn-SOD activities and erythrocyte Se-GPx activities observed for the exposed groups were statistically very significantly higher than in the control group ($p<0.001$). The activities of erythrocyte CAT activities and erythrocyte MDA levels in the exposed groups were found statistically significantly lower than in the control group $p<0.05$, $p<0.001$ respectively. It was found that the mean values of MN frequency in the exposed group were significantly ($p<0.05$) higher than in the control group. There was no significant change found in the frequencies of SCE in exposed group versus controls ($p>0.05$). In conclusion, it has been found a stimulant effect of chronic low-dose radiation in antioxidant protection of exposed individuals and an enhanced resistance to oxidative stress. However, it is concluded that it is necessary of following doses recorded by Thermoluminescence Dosimetry (TLD), taking regularly antioxidant supplementation for protective prevention by exposed individuals. Our findings showed that the increased frequencies of MN in radiology staff indicate the cumulative effect of low-level chronic exposure to ionizing radiation.

PS 325 GENOTOXICITY TESTING OF GATIFOLIA (*GUM GHATTI*).

C. A. Hobbs[1], C. Swartz[1], R. Maronpot[2], J. Davis[3], M. Prajapati[1], K. Shepard[1], P. Allen[1], C. Lentz[1], P. Garibaldi[1], L. Recio[1] and S. Hayashi[4]. [1]*Genetic and Molecular Toxicology, ILS, Inc., Research Triangle Park, NC,* [2]*Maronpot Consulting LLC, Raleigh, NC,* [3]*Investigative Toxicology, ILS, Inc., Research Triangle Park, NC and* [4]*San-Ei Gen F.F.I., Inc., Osaka, Japan.*

Gum ghatti (product name, Gatifolia), a substance derived from the guar plant, is a common food additive in many parts of the world, serving as an emulsifier, a stabilizer and a thickening agent. To evaluate the genotoxic potential of gum ghatti, we conducted GLP-compliant *in vitro* and *in vivo* studies in accordance with the OECD guidelines. Gum ghatti was tested for mutagenicity in a bacterial reverse mutation (Ames) assay. No evidence of toxicity or mutagenicity was noted in a 5-strain (TA97a, TA98, TA100, TA1535, *E. coli* WP2 *uvrA* pKM101) Ames assay testing up to 6.21 mg/plate, either with or without metabolic activation. Gum ghatti was then tested for the ability to induce chromosome structural damage in a chromosome aberration assay using Chinese hamster ovary cells. The results of this test are under analysis and will be presented. To assess the ability to induce DNA damage in a rodent model, we conducted a combined micronucleus (MN)/Comet assay in 8-9 week old male B6C3F1 mice. Gum ghatti was administered at 1000, 1500, and 2000 mg/kg by gavage once daily for 4 consecutive days, 24 hrs after the prior dose, with the exception of the final dose which was administered 18 hr following the previous dose. Samples were collected 4 hr after the final dosing for measurement of micronucleated reticulocyte (MN-RET) frequencies in blood by flow cytometry, and assessment of DNA damage in blood and liver using the Comet assay. No effect of gum ghatti on MN-RET frequency was detected in treated mice. Likewise, no evidence of DNA damage was observed in blood leukocytes or liver as assessed by the % tail DNA endpoint of the Comet assay. Together, our results of both *in vitro* and *in vivo* assays show no evidence of genotoxic potential of gum ghatti administered under the conditions tested. This work was funded by San-Ei Gen F.F.I., Inc.

PS 326 COMPARISON OF TWO DIFFERENT TEST PROTOCOLS FOR AN ACCURATE ASSESSMENT OF THE *IN VITRO* MICRONUCLEUS TEST IN HUMAN LYMPHCYTES.

S. Bohnenberger, A. H. Poth, C. Hall and W. Voelkner. *Genetic and Alternative Toxicology, Harlan Cytotest Cell Research GmbH, Rossdorf, Germany.*

The micronucleus assay in human lymphcytes was developed as a short term screening test for detection of both clastogenic and aneugenic chemicals. For human lymphocytes it is recommended to score micronuclei by the cytokinesis block (CB) method using cytochalasin B. The original method developed by Fenech and Morley, 1985, focuses exclusively on binucleated cells. With respect to the In Vitro Mammalian Cell Micronucleus Test (MNvit) aneugenic and clastogenic reference chemicals (colcemid/demecolcin, mitomycin C and cyclophosphamide) were tested for their potential to induce micronuclei in cultured human peripheral blood lymphocytes. The protocol given by the current OECD Guideline 487 was compared to a slightly modified protocol allowing the cells to have a recovery period between end of treatment and addition of the cytokinesis blocker cytochalasin B. Lymphocyte proliferation was studied in response to stimulation with phytohemagglutinin and the normal cell cycle time was assessed by bromodeoxyuridine uptake. The presented data show difficulties in the detection of an

aneugenic potential in binucleate cells and marked differences in the induction of micronuclei in the micronucleus in vitro by the two different test protocols employed by using the four different reference chemicals.

PS 327 TOXICOLOGICAL EVALUATION OF COMPLEX MIXTURES FROM RESIDUES USED ON AGRICULTURAL LAND APPLICATION.

A. Pillco[1, 2], E. de la Peña[1] and M. Hazen[3]. [1]*Centro de Ciencias Medioambientales, Consejo Superior de Investigaciones Científicas, Madrid, Madrid, Spain,* [2]*Facultad de Bioquímica, Universidad Mayor de San Andrés, La Paz, La Paz, Bolivia and* [3]*Departamento de Biología, Universidad Autónoma de Madrid, Madrid, Madrid, Spain.* Sponsor: E. Vilanova.

Toxicological investigation of complex mixtures (CM) is one of the main focuses of the recent research in toxicology. To study the toxic effects of CM is important because could be hazardous to human and environmental health. Sewage sludge is a residue of the wastewater purification process and it is considered a CM. In the last decades this residue is used for farmland. The aim of this study was to evaluate the ecotoxic and genotoxic effect produced by two different types of CM from sewage sludge used on agricultural land: extract of sewage sludge from municipal wastewater treatment and pirolisis liquid from sewage sludge. The bioluminescence Microtox test (*Vibrio fischeri*), and emergence and growth assays with cress (*Lepidium sativum* L.) were performed to asses ecotoxicity. The results for both assays revealed high toxicity of pirolisis liquid samples. Two short-term bioassays were employed for to evaluate the genotoxicity. First we used the Ames (*Salmonella/microsome* mutagenicity test. The protocol was followed, using tester strains TA98, TA100, TA102 and TA104 of *Salmonella typhimurium*. The results showed presence of mutagenic activity produced by pirolisis liquid. Somatic mutation and recombination test (SMART) in (*Drosophila melanogaster* was employed like a second genotoxic bioassay. 72-h-old larvae were treated at different concentrations of the samples for a period of 48h. Wing of the emerging adult flies were scored for the presence of spots of mutant cells. The results showed positive results for both samples. The four models are rapid, sensitive and economical and they are suitable for testing single compounds and complex mixtures. In conclusion these alternative methods to animal testing demonstrate the existence of ecotoxic and genotoxic risk produced by complex mixtures applied on farmland. Project OTT2007X1317, *MAEC-AECID

PS 328 CYTOTOXIC AND GENOTOXIC EFFECTS OF MAGNETITE ON HUMAN A549 LUNG CELLS *IN VITRO* – INFLUENCE OF ROS, JNK, AND NF-κB.

M. Könczöl[1], V. Mersch-Sundermann[1], S. Ebeling[2], E. Goldenberg[3], B. Grobéty[4], R. Gieré[3], I. Merfort[2], B. Rothen-Rutishauser[5] and R. Gminski[1]. [1]*Department of Environmental Health Sciences, University Medical Center Freiburg, Freiburg, Germany,* [2]*Department of Pharmaceutical Biology, University Freiburg, Freiburg, Germany,* [3]*Department of Geosciences, University Freiburg, Freiburg, Germany,* [4]*Institute of Geosciences, University Fribourg, Fribourg, Switzerland and* [5]*Institute of Anatomy, University Bern, Bern, Switzerland.* Sponsor: J. Pauluhn.

Magnetite, a ferromagnetic iron oxide, has been characterized as a major component of particulate matter sampled at underground stations, along railway lines and at welding workplaces. Furthermore, high amounts of magnetite were found in toner powder. The abundance of magnetite in these samples and the lack of consistent data demand more systematic investigation. In the present study, we studied magnetite for its toxic, especially genotoxic potential in cultured human lung cells (A549). Four fractions relevant for human exposure were investigated: bulk (<10 μm), respirable (2-3 μm), alveolar (0.5-1 μm) and nano-fraction (60 nm). Cell viability (WST-1, NR assay), oxidative stress (DCFH-DA) and genotoxicity (comet assay; CB-MNvit test) were determined as biological endpoints of particle exposure. Additionally, effects on NF-κB and JNK activation were evaluated by EMSA and western blots. TEM images showed incorporation of magnetite bulk and nanoparticles into A549 cells. Slight cytotoxic effects were observed after exposure of the cells to the various samples. We detected size and concentration-dependent induction of micronuclei (MN), as well as DNA-damaging effects. These genotoxic effects could be reduced by the ROS scavengers N-acetylcysteine and butylated hydroxyanisole. Moreover, an activation of JNK without increased NF-κB-binding activity was observed. However, activation of JNK seems to be not ROS dependent. This is the first study to observe increased MN abundance after exposure to magnetite fractions of varying size. Furthermore, these experiments suggest that ROS formation may play an important role in the genotoxicity of magnetite in A549 cells.

PS 329 THE PROTECTIVE EFFECT OF TROLOX AND RESVERATROL ON DNA DAMAGE INDUCED BY CYCLOPHOSPHAMIDE AND TAMOXIFEN IN HUMAN LEUKOCYTES.

R. Naravaneni, S. Sharma, D. Bhaumick and R. D. Mehta. *Genetic Toxicology, PBR Laboratories Inc., Edmonton, AB, Canada.*

Antioxidants like Resveratrol and Trolox (derivative of vitamin E) are well known free radical scavengers. Tamoxifen (TAM) and Cyclophosphamide (CP) are used for the treatment of various types of cancers, but both the drugs also induce severe toxicities by oxidative stress resulting in DNA damage. We investigated the effect of Resveratrol and Trolox on DNA-damaging potential of TAM and CP in human leukocytes using the Comet assay, measuring the response as Tail DNA%. Blood samples from healthy volunteers were pre-exposed for 1 hr at 37°C to Trolox and Resveratrol in two sets (A and B), each at three concentrations of the two antioxidants. Then, to each concentration of set A containing either Resveratrol or Trolox (5, 10 and 20 μM), TAM was added at 10 μM. Similarly, each concentration of set B (90, 180 and 360 μM) received 180 μM of CP. Both sets were then incubated for an additional 1 hr at 37°C in the presence of external metabolic activation (S9). The Resveratrol-CP treatment showed a significant increase in DNA damage at all three concentration of the antioxidant, whereas, cells exposed to Resveratrol-TAM, exhibited a significant dose-related reduction in DNA damage compared to the damage induced by CP alone. Trolox-CP treatment elicited a significant reduction in the CP-induced DNA damage at all three concentrations of Trolox. Similarly, there was a dose related reduction in DNA damage in cells exposed to Trolox-TAM. In conclusion, Trolox was effective in protecting the DNA against the damage induced by CP as well as TAM. Resveratol, on the other hand, was effective in reducing the DNA damage induced by TAM and had no protective effect on CP-induced DNA damage. The results suggest that protective effect of antioxidants on DNA is variable and may depend upon the nature of the interactions between an antioxidant and DNA damaging agent as well as the processes involved in induction and repair of DNA damage.

PS 330 EVALUATION OF THE GENOTOXICITY OF 1, 2-DICHLOROETHANE (EDC).

D. O. Johns[2], M. R. Gwinn[1], T. F. Bateson[1] and K. Z. Guyton[1]. [1]*Office of Research & Development, U.S. EPA, Washington, DC and* [2]*Office of Research & Development, U.S. EPA, Research Triangle Park, NC.*

1,2-Dichloroethane (EDC, CAS#107-06-2) is a high production volume halogenated aliphatic hydrocarbon that has been found in ambient and residential air samples, as well as in groundwater, surface water and drinking water. Target tissues of EDC carcinogenicity in rodents include lung, liver, mammary gland, bile duct, and forestomach of both sexes, as well as uterine and testes. As part of an evaluation of the carcinogenic mechanisms relevant to the human health risks of EDC, we critically evaluated the published data on the genotoxicity data of EDC and its metabolites. The genotoxicity of EDC has been well-studied in a variety of assays, and appears to involve the metabolic activation of the parent compound. EDC is genotoxic in multiple test systems via multiple routes of exposure. EDC has been shown to induce DNA adduct formation, gene mutations and chromosomal aberrations in the presence of key activation enzymes (including CYP450s and/or GSTs) in laboratory animals and in vitro studies. EDC was negative for clastogenesis as measured by the micronucleus assay in mice. In general, an increased level of DNA damage is observed related to the GSH-dependent bioactivation of EDC. Data support a suggestive of a role for the oxidative metabolites of EDC in inducing chromosomal damage. Taken together, our exploratory evaluation of the data suggests that EDC exposure in the presence of key enzymes (including CYP450s and/or GSTs) leads to mutagenicity through DNA adduct formation, gene mutations and chromosomal aberrations. DISCLAIMER: The views expressed are those of the authors and do not necessarily represent the views and/or policies of the U.S. Environmental Protection Agency.

PS 331 INTEGRATION OF PIG-A, MICRONUCLEUS, CHROMOSOME ABERRATION, AND COMET ASSAY ENDPOINTS INTO RODENT TOXICITY STUDIES WITH 4-NITROQUINOLINE-1-OXIDE (4NQO): EFFECT OF DOSE FRACTIONATION ON GENOTOXIC RESPONSES.

L. F. Stankowski, D. J. Roberts, H. Chen, T. E. Lawlor, M. McKeon, H. Murli, A. Thakur and Y. Xu. *Covance Laboratories, Inc., Vienna, VA.*

As part of a multi-lab validation, we examined induction of Pig-a mutant red blood cells (RBCs) and reticulocytes (RETs) by flow cytometry (FCM) during a 28-day subchronic study in male Sprague-Dawley rats using 4NQO (1.25, 2.50, 3.75,

5.00 and 7.50 mg/kg/day by oral gavage; the high dose group was sacrificed on Day 15 due to excessive morbidity/mortality). Animals also were evaluated for micronucleated RETs (mnRETs) by FCM, DNA damage using the comet assay (blood, liver, stomach), and chromosome aberrations (CAbs) in peripheral blood lymphocytes (PBLs). All endpoints were analyzed at two or more timepoints where possible. Statistically significant ($p < 0.05$), dose-dependent increases were observed for: Pig-a mutant RBCs at 7.50 mg/kg/day on Day 15 and 5.00 mg/kg/day on Day 29; Pig-a mutant RETs at ≧5.00 mg/kg/day on Day 15 and ≧2.50 mg/kg/day on Day 29; and mnRETs at ≧2.50 mg/kg/day on Day 29. Responses were consistently higher at the later timepoints, indicating accumulation of genetic damage with repeat dosing. The largest increases observed (fold-increase compared to control, where significant; all at 5.00 mg/kg/day on Day 29) were: mutant RETs (21X)>mutant RBCs (9.0X)>mnRETs (2.0X). In contrast, no such increases were observed for CAbs in PBLs (Days 4 and 29), DNA damage in liver (Days 15 and 29) or stomach (Day 29), or DNA damage in PBLs (Days 1, 15 and 29). Since 4NQO is a well known mutagen, clastogen and carcinogen, the lack of response for some of the endpoints was unexpected. A follow-up study is in progress to evaluate the same endpoints using single acute doses equal to the 28-day cumulative totals, or using the same total doses split over three days as in a typical Comet/micronucleus combination assay. The acute treatments were well tolerated, and half of the treated animals will be followed for up to 28 days after treatment to evaluate persistence of any observed responses.

PS 332 CASE STUDY ON DOSE-RESPONSES FOR MUTAGENICITY AND CLASTOGENICITY INDUCED BY DNA-REACTIVE CHEMICALS.

L. H. Pottenger[1], M. M. Moore[2], T. Zhou[3] and E. Zeiger[4]. *[1]The Dow Chemical Company, Midland, MI, [2]U.S. FDA/NCTR, Jefferson, AR, [3]U.S. FDA/CVM, Rockville, MD and [4]Errol Zeiger Consulting, Chapel Hill, NC.*

Genotoxicity is a key regulatory driver in selecting the dose-response model used for a cancer risk assessment, especially for chemicals acting by a mutagenic mode of action (MOA). Debate continues over the utility of data to inform the shape of low-dose dose-response curves. In 2009, NAS recommended a linear dose-response model as the most likely best fit to describe both cancer and non-cancer data due to human population variability. In recent years, published examples of directly DNA-reactive chemicals with non-linear dose-response curves for genotoxicity have increased. A multi-stakeholder effort was initiated to evaluate published data for genotoxic dose-response characterization using in vitro and in vivo studies of several directly DNA-reactive chemicals: ethylene oxide, ethyl- and methyl methanesulfonate, ethyl- and methylnitrosourea, and acrylamide(/glycidamide). This review, based on a mutation MOA (Pottenger & Gollapudi, 2010), focused on biomarkers of exposure (DNA adducts) and effect (mutations; micronuclei). Such surrogate reporter effects (e.g., Hprt, micronuclei), designated as biomarkers of early effect and not on a path leading to cancer, are nevertheless useful to inform the dose-response of a chemical with a mutagenic MOA. The review concludes that, while the strength of the evidence varies among the chemicals, in part due to methodological issues or inadequate dose coverage, as a whole there is a solid database, one which includes statistical determination of best fit dose-response models in some cases, supporting the conclusion that directly DNA-reactive chemicals can demonstrate non-linear/threshold dose-response relationships for induction of mutation and/or clastogenicity. This analysis provides significant support to the conclusion that, for some carcinogens where the induction of mutation is an early key event (directly DNA-reactive, mutagenic MOA), the overall dose-response for a mutagenic MOA for cancer can also be non-linear. (Does not represent FDA policy.)

PS 333 HIGH-THROUGHPUT *IN VITRO* MICRONUCLEUS ASSAY: AUTOMATED SCORING OF 384-WELL PLATES.

S. Bryce, S. Avlasevich and S. Dertinger. *Litron Laboratories, Rochester, NY.*

This laboratory has described a flow cytometric method for scoring *in vitro* micronuclei (MN) [Environ. Molec. Mutagen. (2006) 47:56-66]. This *In Vitro* MicroFlow® method labels necrotic and mid-/late-stage apoptotic cells with ethidium monoazide. Cells are then washed, stripped of their cytoplasmic membranes, and incubated with RNase plus a pan-nucleic acid dye. Here, we report modifications to the method whereby all procedures are accomplished in the same 384-well plate. For these experiments, CHO-K1 or human TK6 cells were treated continuously for 24 - 30 hrs with a diverse range of genotoxic and non-genotoxic chemicals (51 chemicals tested in CHO, 29 in TK6). For these experiments, each plate included 7 compounds at 20 concentrations in duplicate. With the assistance of a high throughput sampler flow cytometric analyses occurred automatically (2,500 cell equivalents per well). Since a consistent number of latex particles were added to each specimen, nuclei to bead ratios were collected concurrently with MN frequencies, and these data were used to derive relative survival measurements. Dose-dependent MN induction was observed for 27 of the 28 genotoxic agents studied in CHO, and 22 of the 23 genotoxicants studied in TK6. Two instances of false-positive results were observed with the non-genotoxicants. These data were collected at a rate of up to 5 plates, or 1920 wells, per day. Collectively, these data suggest that *in vitro* MN screening can be accomplished in a miniaturized and highly automated format. Further work is needed to characterize assay sensitivity and specificity with a greater number of chemicals, and also to assess the transferability of the method through inter-laboratory trials.

PS 334 PHOTO-CLASTOGENICITY TESTING IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES.

S. Roy, H. Murli, M. Chan, L. F. Stankowski Jr. and T. Lawlor. *Genetic & Molecular Toxicology, Covance Laboratories Inc., Vienna, VA.* Sponsor: M. McKeon.

UV light is known to activate certain classes of chemicals to forms that can be clastogenic to living cells. In order to detect such chemicals, photo-clastogenicity assays are routinely performed using various transformed cell lines. However, transformed cell lines are genetically unstable by nature and can affect the sensitivity and specificity of the assay since many of their cellular functions are compromised as compared to normal primary cells. Therefore, we have evaluated the use of primary human peripheral blood lymphocytes (HPBLs) from healthy donors for this purpose. Venous blood was collected and isolated lymphocytes were PHA-stimulated to grow in mass culture for 48 hours. Individual 10 mL cultures were prepared and treated in 100-mm dishes with 8-methoxypsoralen (8-MOP; 0.10-0.60 μg/mL), or the saline and mitomycin C (0.50 μg/mL) controls. One set of cultures was exposed to a Xenon arc solar simulator lamp with a UV filter (290 nm cut off), while the other set was kept in dark. UV doses ranged from 106 mJ/cm2 - 211.9 mJ/cm2 for UVA and 12.3 mJ/cm2 - 24.7 mJ/cm2 for UVB. All cultures were then incubated in the dark for 3 hours, after which treatments were terminated and cells were grown in fresh culture medium for another 20 hours. Cells were harvested and slides prepared, and chromosome aberrations were analyzed as described in OECD testing guideline 473. 8-MOP induced statistically significant (2 to 3-fold) increases in percent aberrant cells in the presence of photoactivation, but no increases were observed in the absence of solar simulated light. These results indicate that photo-clastogenic chemicals can be detected in primary HPBLs. Further validation is ongoing with additional photo-genotoxic chemicals.

PS 335 PCB3 METABOLITES INDUCE SPINDLE ABERRATIONS AND REDUCE TUBULIN POLYMERIZATION.

S. Flor and G. Ludewig. *University of Iowa, Iowa City, IA.*

Recently, semivolatile lower chlorinated biphenyls (PCBs) have been identified as significant air pollutants in inner city air, in indoor air of schools and other buildings from the 1950s - 1970s, and at 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 rats. PCB3 is readily metabolized to mono- and dihydroxy-biphenyls (PCB3-Cat, PCB3-HQ) and further to quinones (PCB3-oQ, PCB3-pQ). We reported that in cells in culture PCB3-HQ induces polyploidization and all 4 metabolites produce micronuclei, with exception of the PCB3-pQ predominantly through chromosome loss. To gain further insight into the mechanism of this damage induction we analyzed mitotic phase distribution and spindle morphology in V79 Chinese hamster lung cells and tubulin polymerisation in vitro. All 4 metabolites induced a significant increase in monoaster formation of the spindle, in the case of PCB3-HQ with outstanding efficacy, i.e. nearly ¾ of the mitotic cells showed monoaster formation at 7.5 μM. PCB3-pQ had a high potency, ¼ of mitosis showed monoasters at 3 μM. PCB3-Cat induced a wide range of spindle disturbances at the highest concentrations tested (10 and 15 μM), including an increase in tripolar meta- and ana-phase mitosis and multipolar spindles, which was also observed in cells treated with its redox-partner PCB3-oQ (10 μM). The tubulin polymerization assay revealed that PCB3-HQ did not interact with tubulin at concentrations up to 500 μM, whereas all other compounds reduced tubulin polymerization by destabilization of the microtubules. Our results suggest that the formation of monoasters could be involved in the extensive tetraploidization observed with PCB3-HQ, whereas aberrant spindle formation and inhibition of tubulin polymerization may play a role in chromosome loss-induction by these PCB3 metabolites. (Supported by NIEHS P42 ES013661 and DOD DAMD17-02-1-0241)

PS 336 EVALUATION OF THE GENOTOXICITY AND MUTAGENICITY OF THE DYE REACTIVE ORANGE 16.

G. R. Oliveira[1], M. B. Zanoni[2] and D. P. Oliveira[1]. [1]*Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto - FCFRP/USP, Ribeirão Preto, São Paulo, Brazil and* [2]*Departamento de Química Analítica, Instituto de Química - UNESP, Araraquara, São Paulo, Brazil.*

Introduction: The fabrics dyeing began thousands of years ago and the commercial availability of dyes is increasingly. In Brazil, textile industry is considered as one of the main economic activities in the country. The dyeing process is one of the key factors in the commercial success of textile products, since consumers are demanding products more resistant to heat, light, perspiration and washing. This fact is relevant, since textile dyes are discharged into the aquatic ecosystem via industrial effluents and potentially expose humans and biota to adverse effects. The dye Reactive Orange 16 (RO16) is widely used during the fibers dyeing process and it has the group sulfatoethilsulfone as reactive and the group azo as cromophore. Objective: Evaluate the mutagenicity and genotoxicity of dye RO16 using the Salmonella mutagenicity with the strains TA98 and TA100 and comet assays in HepG2 cells. Methodology: Comet test was carried out according to Tice et al. (2000) with slight modifications and Salmonella mutagenicity assay was used, with or without S9 metabolic activation, according to Maron and Ames (1983). Different concentrations of the dye RO16 were examined for comet (1.0, 5.0, 10.0, 25.0 and 50.0 μg/mL) and for Salmonella assays (doses at a logarithmic range from 0.5 to 5000 μg/plate). Results: Dye studied induced damage in the DNA of the HepG2 cells in a dose-dependent manner and it also showed positive mutagenic response for TA100 strain with S9 (0.05 revertants per μg). No effect was observed for TA98 with and without S9, as well as for TA100 in the absence of S9. Conclusion: The use of RO16 should be avoided for dyeing textile fibers that will be in contact with skin. Additionally, the treatment of effluent containing this dye should be carefully evaluated in order to prevent harmful effects for human and biota after the exposure to water and food contaminated with this compound.

PS 337 COMPARISON OF ANEUPLOIDIES OF CHROMOSOMES X, Y, AND 21 IN THE PERIPHERAL BLOOD LYMPHOCYTES AND SPERM CELLS OF WORKERS EXPOSED TO BENZENE.

Z. Ji[1], R. H. Weldon[1], F. Marchetti[2], H. Chen[1], G. Li[3], C. Xing[2, 3], E. Kurtovich[1], S. Young[1], T. E. Schmid[2], S. Waidyanatha[4], S. Rappaport[1], A. J. Wyrobek[2], L. Zhang[1] and B. Eskenazi[1]. [1]*School of Public Health, University of California at Berkeley, Berkeley, CA,* [2]*Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA,* [3]*China CDC, Beijing, China and* [4]*National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Benzene, an established human carcinogen, is known to cause aneuploidy in both human blood and sperm cells, but with considerable variation among individuals. However, a comparison of aneuploidies induced by benzene in somatic and germline cells within the same individuals has not been reported. We conducted a cross-sectional study of 33 benzene-exposed male workers and 33 unexposed men from Chinese factories to compare the frequencies of aneuploidy in their white blood cells and sperm. Individual exposures were assessed using personal air monitoring and urinary concentrations of benzene and *trans,trans*-muconic acid (*E,E*-MA). Previously, we reported that benzene exposure was positively associated with sex chromosome gains in sperm (Environ Health Perspect, 2010, 118(6):833-9). In the present study, we examined lymphocyte aneuploidies using blood collected within a few days of when sperm samples were provided. Stimulated peripheral blood was cultured for 72 hrs and metaphases were examined by multicolor fluorescence *in situ* hybridization with probes for chromosomes X, Y, and 21. Among exposed workers, log-transformed urinary *E,E*-MA concentrations were positively associated with sex chromosome loss in lymphocytes, in particular with loss of chromosome X (β=0.28, p=0.01). This finding of loss of sex chromosomes in lymphocytes contrasts with the gain of sex chromosomes in sperm from the same group of workers. Furthermore, we found that the chromosome-specific frequencies of aneuploidies in lymphocytes were not correlated with those in sperm. Our findings show that occupational benzene exposure induced aneuploidies in both blood and sperm cells, and suggest that the mechanisms of aneuploidy induction are different between somatic and germ cells.

PS 338 *IN VITRO* GENOTOXIC MECHANISMS OF ARSENIC TRIOXIDE IN HUMAN HEPATOCELLULAR CARCINOMA (HEPG2) CELLS: INVOLVEMENT IN OXIDATIVE STRESS.

E. T. Brown, C. G. Yedjou and P. B. Tchounwou. *Jackson State University, Jackson, MS.*

Recent studies in our laboratory indicated that oxidative stress plays a key role in arsenic trioxide (ATO)-induced cytotoxicity in human cancer cells. In the present investigation, we used human hepatocellular carcinoma (HepG2) cells as a model to determine whether arsenic induced DNA damage is mediated through oxidative stress. To achieve this goal, oxidative stress biomarkers were measured by lipid peroxidation, glutathione peroxidase, and catalase assays, respectively. The degree of DNA damage was estimated by the means of comet assay. The results of the lipid peroxidation showed a significant increase ($p < 0.05$) of malondialdehyde (MDA) levels with increasing ATO concentrations. Results from the glutathione peroxidase and catalase assays showed a gradual decrease in antioxidant enzyme activity in ATO-treated cells as compared the control. In regard to the comet assay, a significant increase ($p < 0.05$) in comet tail-length and percentages of DNA cleavage were observed in ATO-treated cells using the comet assay. Taken together, our research demonstrated that oxidative stress modulates ATO mediated DNA damage in HepG2 cells.

PS 339 EFFICIENT SCORING OF MICRONUCLEI AND CELL CYCLE STATUS IN THE RECONSTRUCTED SKIN MICRONUCLEUS (RSMN) ASSAY USING FLOW CYTOMETRY.

M. Crosby[1], E. Dahl[2], S. Bryce[3], A. Allemang[1], S. Brown[1] and S. Pfuhler[1]. [1]*Procter & Gamble Company, Cincinnati, OH,* [2]*Institute for in vitro Sciences, Gaithersburg, MD and* [3]*Litron Laboratories, Rochester, NY.*

The in vitro human reconstructed skin micronucleus (RSMN) assay performed with EpiDerm™ 3D human skin models was designed as an in vitro follow-up tool for measuring the induction of micronuclei (MN) following dermal exposure by compounds that were positive in the in vitro standard battery. However, the manual evaluation of keratinocyte slide preparations is very labor intensive, increasing the cost of the assay. Based on the Litron In Vitro MicroFlow® system, we have investigated the feasibility of scoring keratinocyte preparations using flow cytometry (FC). Using two model compounds, the aneugen, vinblastine (VB) and the clastogen, cyclophosphamide (CP), we established a new protocol enabling sensitive detection of MN and cell cycle status. The scoring of MN post-CP treatment in skin models is difficult to detect in the standard RSMN assay [48 h exposure in the presence of cytochalasin B (cyto B), manual evaluation]. However, when a 96 h protocol (non-cyto B) was used, a 6-fold increase in MN was detected with respect to the solvent control by FC. Similarly, a >5-fold increase in MN was detected in tissues treated with VB (0.0015 μg/tissue) with respect to the solvent control. Notably, genotoxicity at this dose as measured by the standard protocol is undetectable. Thus, the FC method appears to enhance assay sensitivity and leads to superior statistical power, as 30x more nuclei may be scored. Although cyto B allows for the tracking of cell division, cell cycle regulation may also be measured via propidium iodide (PI) staining. Using this method, we detected a strong G2/M-phase arrest induced by VB (up to 8-fold increase in the percentage of cells in G2/M) and a moderate (< 3-fold) G2/M-phase arrest with CP at 96 h.
The results of this study indicate that the described methods simplify the evaluation of micronuclei in the RSMN, increase its sensitivity and statistical power, and allow for generation of valuable cell cycle information.

PS 340 NEXT GENERATION DEL ASSAY: RAPID AND ACCURATE ASSESSMENT OF TOXICITY.

Y. Rivina and R. H. Schiestl. *Environmental Health Sciences, University of California, Los Angeles, Los Angeles, CA.*

Genetic instability and DNA deletions are involved in carcinogenesis. Furthermore, cells from patients carrying mutations conferring cancer-prone phenotypes show a higher level of genetic instability including DNA deletions. We have constructed and used assays (DEL assays) that select for DNA deletion events in yeast, human cells and in vivo in the mouse. Deletions occur by homologous recombination between two copies of a duplicated DNA sequence to delete the intervening sequence together with one repeat. 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. Furthermore, many cancer prone mutations highly elevated the frequency of DNA deletions in mice adding to the solid correlation between DEL

events and carcinogenesis. Here we introduce the next generation of HTS methodology: a novel dual-read out Saccharomyces cerevisiae screen DEL-XG. Based on the DEL assay, DEL-XG simultaneously assesses the compound's genotoxicity and cytotoxicity properties. DEL-XG test strain cells contain a lacZ gene sequence (with a GAL promoter) with a region of homology interrupted by a URA3 gene; during an exposure to a genotoxic event (carcinogens, ionizing radiation, etc) the URA3 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 detectable indole product. Additionally, the test strain stably expresses a florescent protein as an indication of survival. Thus, in a single well the assay identifies how the compound of interest affects survival—changes in florescence emission, and genetic integrity—accumulation of insoluble blue dye. DEL-XG is a rapid and economical way to screen large chemical libraries for toxicity properties and enables a screening "triage": compounds with higher toxicity profiles will be subjected to additional screening as a first priority.

PS 341 THE RECONSTRUCTED SKIN COMET ASSAY SHOWS GOOD INTRA- AND INTER-LABORATORY REPRODUCIBILITY: RESULTS FROM THREE LABORATORIES.

T. Downs[1], A. Reus[2], K. Reisinger[3], C. Krul[2] and S. Pfuhler[1]. [1]*Procter & Gamble Company, Cincinnati, OH,* [2]*TNO Quality of Life, Zeist, Netherlands and* [3]*Henkel AG & Co KgaA, Düsseldorf, Germany.* Sponsor: G. Daston.

EU policy marks the need for a broad replacement of animal tests in relation to the 7th Amendment to the Cosmetics Directive. For cosmetics ingredients as well as many chemicals, the skin is the main route of exposure. The currently used standard in vitro genotoxicity assays, if used in a test battery approach, show a high percentage of irrelevant positive results. Moreover, there is no follow-up assay available in the target tissue skin and the use of in vivo assays is not allowed to further evaluate a positive response obtained from the in vitro assays. Genotoxicity assays using reconstructed human skin could however serve this purpose. EpiDerm™ skin models were topically exposed to vehicle or test compounds for 3h, followed by cell isolation and measurement of DNA damage using the Comet assay methodology. Inter-laboratory reproducibility of the 3D skin Comet assay was demonstrated for MMS and 4NQO and results showed good concordance with in vivo data. In the next phase of the project, the assay was performed with 3 coded compounds shipped to 3 different laboratories. The results of the collaborative study showed good reproducibility within and between laboratories. However, considerable intra- and inter-experimental variability was often observed in the background level of DNA damage in 3D skin tissues, with solvent control values sometimes greater than 40%. To resolve these problems we worked on several aspects of the experimental procedure and promising results were seen with the use of "underdeveloped" tissues, which are shipped at an earlier stage (5 days before regular shipment) and are then matured in our laboratories. Results thus far indicate that the comet assay in reconstructed 3D skin models is a relevant model for the safety assessment of chemicals with dermal exposure. This work was sponsored by the European Cosmetics industry (COLIPA) and the European Center on Validation of Alternative Methods (ECVAM)

PS 342 CHLORPYRIFOS DOES NOT INDUCE RAT BRAIN DNA DAMAGE OR BONE MARROW MICRONUCLEUS FORMATION IN A MICRONUCLEUS AND COMET COMBINATION ASSAY.

Y. Xu, K. Carl and L. Timothy. *Covance, Vienna, VA.*

Chlorpyrifos was reported to induce single strand DNA breakage in the brain of rats, but but was not reported to be mutagenic in WHO and FAO evaluations. A combined micronucleus and Comet assay using Cyclophosphamide (CP) and Ethyl Methanesulfonate (EMS) as a positive control was performed to confirm the findings. Differing Comet assay conditions (unwinding time and electrophoresis time) were tested to verify the reported data. As demonstrated in the mouse micronucleus assays, Chlorpyrifos did not induced bone marrow micronucleus formation at doses up to 100 mg/kg in three doses. The treatment of Chlorpyrifos did not induce an increase of % Tail Intensity or Tail Moment in the cerebral cortex cells of the brain as published. However, CP/EMS treatment significantly elevated the % Tail Intensity and Tail Moment in the Comet assay. The negative findings with Chlorpyrifos were further confirmed by the differing Comet assay conditions. When unwinding and electrophoresis times were increased from 15/20 minutes to 30/40 minutes, the group mean of % Tail Moment in positive control animals was increased about 6 fold from 1.35 ± 0.19 to 3.03 ± 1.36. Meanwhile the fold increases in Chlorpyrifos groups (3 fold at 100 mg/kg and 2.3 fold at 50 mg/kg) approximated the vehicle control group (2.7 fold). The data supports a non-genetoxic evaluation of Chlorpyrifos and did not confirm DNA damage effects of Chlorpyrifos in the cerebral cortex cells of brain.

PS 343 GENOTOXICITY TESTING USING THE MICRONUCLEUS AND COMET ASSAYS IN NORMAL HUMAN CELL BASED 3D EPITHELIAL MODELS.

J. DeLuca, Y. Kaluzhny, P. J. Hayden, A. Armento, V. Karetsky and M. Klausner. *MatTek Corp, Ashland, MA.*

Safety assessment of new products for human use requires genotoxicity testing to ensure non-carcinogenicity. Current in vitro assays have low specificity resulting in a high rate of false positives. To determine the biological relevance of positive in vitro genotoxicity results, in vivo assays are conducted. However, in vivo genotoxicity testing was banned in 2009 by the 7th Amendment to the Cosmetics Directive. 3D human tissue models, which have in vivo-like barrier function and metabolism and which allow for topical exposure, are predicted to have improved biological relevance. Toward this end, the Reconstructed Skin Micronucleus (RSMN) and Comet assays (CA) that utilize MatTek's highly differentiated EpiDerm™ tissue model have been adapted for use with tracheal, vaginal, oral, and corneal tissues. EpiDerm is a 3D normal human cell-based epidermal model that is highly reproducible, contains an in vivo-like barrier, and possesses in vivo-like biotransformation capabilities. RSMN assay results show statistically significant dose-dependent, increases in cells containing micronuclei (MNC) for 9 direct genotoxins and 6 genotoxins that require metabolic activation, and no increases for 4 non-genotoxins. In addition, CA results show statistically significant increases in % tail DNA after treatment with a model genotoxin. Utilizing the RSMN protocol with tracheal, vaginal, oral, and corneal tissue models, statistically significant increases in MNC (0.3 to 1.2%) were observed after treatment with genotoxins. Similarly, CA results with tracheal, vaginal, oral, and corneal tissue models showed statistically significant increases in % tail DNA. Hence, the EpiDerm RSMN and CA assays can be applied to other in vitro human epithelial tissue models to predict genotoxic effects following real life exposure conditions. Together, RSMM and Comet assays for skin, tracheal, vaginal, oral, and corneal tissue models will identify a wide spectrum of genotoxic hazards and will increase confidence in the veracity of in vitro test results.

PS 344 COLOCALIZATION OF CANNABINOID RECEPTOR SUBTYPES IN CULTURED RAT CORTICAL ASTROGLIAL CELLS.

J. A. Torres[1, 2] and A. Shivachar[2]. [1]*Environmental Toxicology, Texas Southern University, Houston, TX and* [2]*College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX.*

Discovery of the cannabinoid (CB) receptor subtypes opened a door to our understanding of Marijuana-derived cannabinoids action. So far, there are two well-known subtypes of cannabinoid receptors: CB1 expressed in the brain and in other tissues, and CB2 expressed mainly in immune cells and brain microglia. Although the expression of CB1 in astrocytes cells is well established, the expression of CB2 receptors remained somewhat controversial. Since previous studies show a partial antagonism by CB1 selective SR141716A, it's speculated that a second CB-like receptor may exist in these cells. Therefore we hypothesize that CB1 and CB2 are co-expressed in astroglial cells. Highly enriched rat cortical astrocyte cultures, devoid of microglial cells, were prepared by shaking and low-density plating. The resulting protoplasmic astrocytes expressed glial fibrillary acidic protein (GFAP) and CB1 receptors. Similarly, cells that expressed GFAP were also stained positive for CB2 receptor. The secondary antibody controls showed no detectable fluorescence indicating signal-specificity. Furthermore double labeling studies using rabbit anti-CB1 and mouse anti-CB2 antibodies indicated that CB1 and CB2 receptors are co-localized in the same astroglial cell. Western blot analyses corroborated our immunocytochemistry results and showed distinct immunoreactive bands at the molecular mass of CB1 (62 KDa) and CB2 (42KDa). Preincubation with CB2 blocking-peptide, completely abolished the CB2 immunoreactivity. Together, these results indicate that astroglial cells constitutively coexpress CB1 and CB2 receptors. Thus, by coexpressing CB1 and CB2 receptor subtypes, astrocytes may represent a unique in vitro model system in which to study the CB1 and CB2 receptor cross-talk on neuro- and immuno- modulatory responses of Marijuana-derived neurotoxicants (Funding support NCRR-RCMI#G12RR03045-17 and NASA JPFP fellowship).

PS 345 PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR γ IS ACTIVATED BY 15-DEOXY-PGJ$_2$-G, A PUTATIVE METABOLITE OF THE ENDOCANNABINOID, 2-ARACHIDONYL GLYCEROL.

P. Raman[1, 2], B. Kaplan[1, 2], J. Thompson[3], J. Vanden Heuvel[3] and N. Kaminski[1, 2]. [1]*Pharmacology & Toxicology, Michigan State University, East Lansing, MI,* [2]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [3]*Veterinary Science & Biomedical Sciences, Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA.*

2-Arachidonyl glycerol (2-AG), an endocannabinoid, suppressed IL-2 secretion in activated T cells by the activation of peroxisome proliferator activated receptor γ (PPARγ) independently of the cannabinoid receptors, CB1 and CB2. One of the putative metabolites of 2-AG is a glycerol ester of a prostaglandin, 15-deoxy prostaglandin J$_2$-glycerol (15d-PGJ$_2$-G). Since one of the known ligands for PPARγ is 15-deoxy-prostaglandin J2 (15d-PGJ$_2$), 15d-PGJ$_2$-G was investigated as a putative PPARγ ligand. Previously, it was demonstrated that 15dPGJ$_2$-G transcriptionally activated PPARγ in HEK293T cells and it has been demonstrated that 2-AG and other PPARγ agonists suppress interleukin-2 (IL-2) in activated T cells. Therefore, we investigated if 15d-PGJ$_2$-G activates PPARγ in a T cell line. In Jurkat T-Ag cells transfected with PPARγ reporter, 15d-PGJ$_2$-G treatment induced PPARγ reporter. Time of addition studies using activated Jurkat T cells demonstrated that addition of 15d-PGJ$_2$-G either 30 min before or after Jurkat cell activation produced marked IL-2 suppression whereas addition of 15d-PGJ$_2$-G at later times (1, 2, 4, 8 h post stimulation) resulted in an attenuation of 15d-PGJ$_2$-G-mediated IL-2 suppression. In addition, 15d-PGJ$_2$-G decreased the transcriptional activity of nuclear factor of activated T cells (NFAT). Importantly, 15d-PGJ$_2$-G-mediated IL-2 suppression and the decrease in NFAT reporter activity were attenuated in the presence of a PPARγ antagonist, T0070907. We hypothesize that 15d-PGJ$_2$-G is a metabolite of 2-AG and is responsible for IL-2 suppression through the activation of PPARγ and the decrease in NFAT transcriptional activity. (Support in part by NIH RO1 DA12740 and Pfizer Global Research).

PS 346 PHARMACOLOGICAL STUDY OF SEROTONIN AND DOPAMINE POST-SYNAPTIC RECEPTORS OF THE LATERAL CILIATED CELLS OF THE GILL AND VISCERAL GANGLIA OF *CRASSOSTREA VIRGINICA.*

C. Brown, Z. Bandaogo, E. J. Catapane, M. A. Carroll, A. Hernandez and D. Adebesin. *Biology, Medgar Evers College, Brooklyn, NY.*

Lateral cilia of gill of *Crassostrea virginica* are controlled by serotonergic–dopaminergic nerves from their visceral and cerebral ganglia. Serotonin (HT) and dopamine (DA) are the neurotransmitters that increase and decrease ciliary beating rates, respectively. We undertook a pharmacological study of the HT receptor types. HT receptor families are classified as HT1 - 7, with subtypes. All but HT3 are G protein metabotrophic; HT3 are iontotrophic. DA receptors are classified as D1-like and D2-like, each with respective subtypes. D1 receptors are coupled to G protein Gαs and activates adenyl cyclase. D2 receptors are coupled to the G protein Gαi, and directly inhibits the formation of cAMP by inhibiting the enzyme adenyl cyclase. Beating rates of cilia were measured by stroboscopic microscopy of isolated gill preparations and VG-gill preparations in which the innervation of the gill by the visceral ganglia was kept intact. Agonists and antagonists of different receptor families (5-carboxamidotryptamine, alpha methyl serotonin, BIMU8, 1-(3-chlorophenyl)biguanide methysergide, NAN-190, GR127935 and clozapine N-oxide, A68930, propylpiperdine, piribedil, BHT920, 2-bromo-ergocryptine, SKF89626, supiride, droperidol, metoclopramide, ergonovine and chlorprothixene) were tested at the gill and visceral ganglia to determine their efficacy in altering cilia beating rates. Analysis of the receptor families and subtypes for the agonists and antagonists indicates that HT4 or HT7 receptors are present in the gill and visceral ganglia, and that the DA receptors present in the gill and visceral ganglia are the D2 type. The study also shows that this preparation is a good model for pharmacological studies of serotonin and dopamine function as well as the pharmacology of drugs affecting biogenic amines in nervous systems. This work was supported by 2R25GM06003 of the Bridge Program of NIGMS, 0516041071 of NYSDOE and 0622197 of the DUE Program of NSF.

PS 347 SPECIFIC AMINO ACID RESIDUES WITHIN THE LIGAND BINDING DOMAIN CONFER LOW RESPONSIVENESS TO 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) TO AN ARYL HYDROCARBON RECEPTOR (AHR) FROM THE FROG *XENOPUS LAEVIS.*

C. Odio[1], S. A. Holzman[1], M. S. Denison[2], L. Bonati[3] and W. H. Powell[1]. [1]*Biology, Kenyon College, Gambier, OH,* [2]*Environmental Toxicology, University of California-Davis, Davis, CA and* [3]*Scienze dell'Ambiente e del Territorio, Universita` degli Studi di Milano-Bicocca, Milano, Italy.*

Frogs are remarkably insensitive to TCDD toxicity. Previous studies in *Xenopus laevis* revealed that frog AHRs bind TCDD with low affinity. In other vertebrates, differences in TCDD toxicity and AHR binding affinity result from differences in specific amino acids within the ligand binding domain (LBD). We sought to identify structural determinants within *X. laevis* AHR1β associated with low TCDD responsiveness using transactivation assays. Substitution of the entire LBD with the corresponding sequence from mouse AHR^{b-1} restored TCDD responsiveness. Surprisingly, the frog LBD contains all residues previously associated with high affinity TCDD binding in mouse AHR alleles, suggesting a role for other amino acids in the low-affinity phenotype. To seek additional candidate residues, we constructed a comparative model of the AHR1β LBD using the homologous domains of HIF2α and ARNT. The model revealed an internal cavity similar in dimensions to the putative binding pocket of mouse AHR^{b-1}, suggesting the importance of side-chain interactions over cavity size. Of residues with side chains clearly pointing into the pocket, only two differed from the mouse sequence. When A354, located within a conserved β-strand, was changed to serine, the homologous mouse residue, the EC$_{50}$ for TCDD decreased more than 15-fold. When N325, in a long α-helical connector, was changed to serine, EC$_{50}$ declined 3-fold. The combined mutations resulted in a 22-fold reduction in EC$_{50}$, from 18.75 nM to 0.83 nM, a large but incomplete recovery of TCDD responsiveness. The location of these residues suggests that low responsiveness of AHR1β in transactivation assays is substantially attributable to low affinity binding of TCDD. [NIH ES011130 (WHP), ES07685 (MSD)]

PS 348 THE NOVEL DAPHNIA NUCLEAR RECEPTOR, HR97G, IS ACTIVATED BY JUVENILE HORMONE ANALOGS.

G. Ginjupalli[2], Y. Li[2] and W. S. Baldwin[2, 1]. [1]*Biological Sciences, Clemson University, Clemson, SC and* [2]*Environmental Toxicology Program, Clemson University, Clemson, SC.*

Daphnia magna are a commonly used sentinel specie in toxicity testing. The *Daphnia pulex* genome sequenced recently revealed three novel nuclear receptors related to the CAR/PXR/VDR group and were called as HR97a/b/g, because of their similarity to the HR96 nuclear receptors in *Drosophila* and *Daphnia*. We cloned HR97g from *Daphnia magna*, and then made a Gal4-expression vector containing the LBD of HR97g for transactivation assays. These assays found only three activators, cortisol, pyriproxyfen, and methyl farnesoate; two of which are key juvenile hormone analogs that induce the production of males in the otherwise female parthenogenic *Daphnia*. Dose-response studies found that pyriproxyfen and methyl farnesoate have EC50's of 3.4 and 2.2 uM, respectively. Immunohistochemistry (IHC) revealed that HR97g is primarily expressed in the gut, ovaries, and mandibular organ, a key organ in juvenile hormone production. RT-PCR and IHC demonstrated that HR97g is predominantly expressed in young, pre-reproductive daphnids less than 8 days old similar to juvenile hormone expression. In addition, treatment of adult female *Daphnia magna* with 200 or 400ng/L pyriproxyfen induces HR97g expression providing further evidence that HR97g is associated with juvenile hormone function. In summary, the juvenile hormone receptor is an ongoing search and HR97g is a candidate juvenile hormone receptor in the crustacean, *Daphnia magna*, because it is activated by juvenoids, its expression is associated with juvenoids, and it is induced by juvenile hormone analogs such as the pesticide pyriproxyfen.

PS 349 LIGAND-ACTIVATED AHR ALTERS THE EXPRESSION OF GENES IN THE HUMAN EPIDERMAL DIFFERENTIATION COMPLEX.

C. H. Sutter, S. Bodreddigari, C. Campion, R. Wible and T. Sutter. *Biological Sciences, University of Memphis, Memphis, TN.*

In humans exposed to dioxin (TCDD), the most often observed and best studied toxic response is chloracne. Chloracne is manifested in the skin as hyperkeratinization of the interfollicular squamous epithelium, hyperproliferation and hyperkera-

tinization of cells of the hair follicle, as well as a metaplastic response of the ductular sebaceous glands. Recently, dioxin was shown to enhance human keratinocyte differentiation and to elevate the expression of three genes involved in cornification, filaggrin (FLG), UDP-glucose ceramide glucosyltransferase (UGCG), and sphingolipid C4-hydroxylase/delta 4-desaturase (DEGS2). Of importance, epidermal growth factor (EGF) receptor signaling was shown to block TCDD and aryl hydrocarbon receptor (AHR)-mediated gene expression and cell differentiation. Using chromatin immunoprecipitation assays we determined that in human normal epidermal keratinocytes (NHEKs) TCDD-activated AHR was bound to XREs found upstream of the transcriptional start sites of FLG and DEGS2. Reporter assays using the promoter region of FLG demonstrated that this gene was transcriptionally regulated by TCDD. Moreover, loss of regulation when both XREs were mutated provided strong evidence that this gene is regulated by the AHR. Immunoblot assays demonstrated that profilaggrin protein was increased by TCDD and repressed by EGF. As FLG is part of the human epidermal differentiation complex found on chromosome 1 we investigated 19 other genes in this complex for their regulation by TCDD using RT real-time PCR. Of these genes, 16 were increased by TCDD. The increases by TCDD were repressed by EGF in 13 of the 16 genes. These results will aid in determining the molecular effects of dioxin exposure that manifest in chloracne.

PS 350 ACTIVATING PKCβ1 AT THE BLOOD-BRAIN BARRIER (BBB) REVERSES INDUCTION OF P-GLYCOPROTEIN (PGP) ACTIVITY BY DIOXIN AND RESTORES DRUG DELIVERY TO THE CNS.

X. Wang, B. Hawkins and D. Miller. *Laboratory of Toxicology and Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

The ATP-driven drug efflux pump, Pgp is highly expressed at the BBB, where it is a major impediment to pharmacotherapy of CNS diseases. Therapeutic drugs and environmental toxicants upregulate Pgp expression at the BBB through xenobiotic-activated nuclear receptors. Increased transporter expression is accompanied by selective tightening of the barrier to drugs that are Pgp substrates. Recent studies show activation of protein kinase C isoform β1 (PKCβ1) at the BBB rapidly, reversibly and specifically reduces basal Pgp transport activity in vitro and in vivo. The present experiments are focused on determining whether PKCβ1-based signaling reverses CNS drug resistance caused by arylhydrocarbon receptor (AhR)/dioxin induction of Pgp expression. We measured Pgp activity in freshly isolated rat brain capillaries as specific luminal accumulation of a fluorescent Pgp substrate using confocal microscopy and digital image analysis. Exposing capillaries to the potent AhR ligand, TCDD (0.5nM for 3h) more than doubled Pgp-mediated transport; stimulation was abolished when PKCβ1 was specifically activated using 10 nM 2-deoxyphorbol-13-phenylacetate-20-acetate (dPPA). Dosing rats with 5μg/kg TCDD significantly increased Pgp transport activity and Pgp protein expression in brain capillaries assayed ex vivo. Increased transport activity was abolished when capillaries were exposed to 10 nM dPPA. In situ brain perfusion of rats dosed with 5μg/kg TCDD showed significantly reduced brain uptake of the Pgp substrate, verapamil, when compared to untreated controls. Perfusing the brain vasculature of TCDD-dosed rats with dPPA attenuated the effect of TCDD on the brain uptake of verapamil. Thus, activation of PKCβ1 at the BBB not only reduced basal Pgp activity, but also reversed the increase in activity due to AhR induction of transporter expression. Targeting PKCβ1 may be an effective strategy to improve drug delivery to the brain, even in a population of drug resistant patients.

PS 351 ARYL HYDROCARBON RECEPTOR ANTAGONISTS INHIBIT ADIPOCYTE-BREAST CANCER CELL INTERACTIONS.

T. Salisbury, R. Jones, M. Hodge, K. Geronilla and N. Santanam. *Marshall University School of Medicine, Huntington, WV.* Sponsor: M. Valentovic.

The microenvironment from which a tumor grows plays a major role in tumor initiation and progression. Within the breast, adipocytes are an important component of the tumor microenvironment and through their release of adipokines stimulate breast cancer cell growth. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the effects of environmental pollutants, as well as, endogenous cellular proliferation. Given these roles we hypothesized that AHR antagonists would regulate adipocyte-stimulated increases in breast cancer cell proliferation. We used cell counting to verify that adipocyte-conditioned media is sufficient to stimulate increases in the proliferation of the MCF7 cells, a human breast cancer cell line. We discovered the aryl hydrocarbon receptor antagonists alpha-naphthoflavone (ANF) and resveratrol (Rv) strongly inhibited adipocyte-stimulated increases in MCF7 cell proliferation. Adipokine protein array analysis showed that adipocyte conditioned media in our model system contained several mitogenic factors including the hormones leptin and IGF-1. We used cell counting to discover that ANF strongly inhibits IGF-1 stimulated increases in MCF7 cell proliferation. We then used quantitative real-time PCR (QT-PCR) to show that in accordance with prior reports, IGF-1 stimulated the expression of several cyclins, and that ANF selectively reduced the expression of a subset of these IGF-1 gene targets. Collectively, these data show for the first time that AHR antagonists inhibit adipocyte-breast cancer cell interactions, and suggest this effect, at least in part, maybe attributed to interference with IGF-1 signaling.

PS 352 DUAL PPAR/RXR AGONIST TRIBUTYLTIN UNIQUELY IMPACTS BONE INTEGRITY AND MARROW MICROENVIRONMENT.

A. H. Baker[1], B. D. Meeks[2], K. K. Mann[3], L. C. Gerstenfeld[2] and J. J. Schlezinger[4]. *[1]Medicine, Boston University, Boston, MA, [2]Orthopedic Surgery, Boston University, Boston, MA, [3]Oncology, McGill University, Montreal, QC, Canada and [4]Environmental Health, Boston University, Boston, MA.*

Bone marrow's ability to perform myriad functions, from supporting bone integrity to sustaining hematopoiesis, rests on a balance of stromal elements. There is a reciprocal relationship between commitment of mesenchymal stem cells to osteoblastogenesis or adipogenesis. It is likely that PPARγ, the master regulator of adipocyte differentiation, is a key regulator of the bone/fat balance. Exposure to PPARγ agonists occurs via therapeutic (TZDs) and environmental (phthalates and organotins) sources. Tributyltin (TBT) is a highly potent dual PPARγ/RXRα agonist. We hypothesize that TBT impairs osteoblast differentiation and increases adiposity. Its ability to bind PPARγ and RXRα suggests that TBT would have similar yet distinct effects than either a PPARγ (rosiglitazone) or RXRα (bexarotene) agonist. In vitro studies of a bone marrow stromal cell line (BMS2) and primary mouse bone marrow cultures show that TBT (1-100 nM) increased lipid accumulation and adipocyte differentiation (Nile red staining, perilipin) and suppressed all measures of osteogenesis (alkaline phosphatase activity, nodule number, mineralization). Bex suppressed all measures of osteogenesis without stimulating adipogenesis; rosi dramatically increased adipogenesis while only decreasing alkaline phosphatase activity. TBT, rosi, and bex alter expression of adipocyte-specific genes (PPARγ2 and FABP4) with similar potency but differing efficacy (rosi>TBT>bex) in a PPARγ-dependent manner. We expect they will alter osteoblast-specific gene expression. Long term, low-dose exposure to TBT or rosi in vivo decreased cortical bone (BA/TA). We hypothesize that key osteogenic and adipogenic gene expression in these bones is altered. Analysis of these agonists will augment the understanding of both the impact of environmental and therapeutic PPARγ agonist exposures on bone homeostasis and the contribution of PPARγ and RXRα to bone marrow physiology.

PS 353 MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF ARYL HYDROCARBON RECEPTOR REPRESSOR (AHRR) FROM THE CHICKEN (*GALLUS GALLUS*).

H. Iwata[1], J. Lee[1], E. Kim[2] and K. Nomaru[1]. *[1]Center for Marine Environmental Studies, Ehime University, Matsuyama, Japan and [2]Department of Life and Nanopharmaceutical Science and Department of Biology, Kyung Hee University, Seoul, Republic of Korea.*

The aryl hydrocarbon receptor (AHR) repressor (AHRR) has been recognized as a negative feedback modulator of AHR-mediated responses in fish and mammals. However, the repressive mechanism by the AHRR has not been investigated in other animals. To understand the molecular mechanism of dioxin toxicity and the evolutionary history of the AHR signaling pathway in avian species, the present study addresses chicken AHRR (ckAHRR). The cDNA sequence of ckAHRR encodes an 84 kDa protein sharing 29-52% identities with other AHRRs. High levels of ckAHRR mRNA were recorded in the kidney and intestine of non-treated chicks. In both chicken embryo livers and hepatoma LMH cells, the 2,3,7,8-TCDD-EC_{50} value for ckAHRR induction (0.0016 nM) was the same as that for chicken CYP1A5 (ckCYP1A5), implying a shared transcriptional regulation of ckAHRR and ckCYP1A5 by AHR. In ckAHRR transient transfection assays, ckAHRR repressed both AHR1- and AHR2-mediated transcriptional activities. Deletion and mutation assays revealed that bHLH-PAS A domains of ckAHRR, particularly 217-402 amino acid residues are indispensable for the repression, but the ARNT sequestration by ckAHRR and SUMOylation of ckAHRR are not involved in its repressive mechanism. Additionally, subcellular localization assay of ckAHR1-EGFP fusion protein showed that ckAHRR did not affect nuclear translocation of the ckAHR1. Furthermore, ckAHRR inhibited the enhanced

CYP1A transcription mediated by AHR-estrogen receptor α (ERα) crosstalk. Taken together, the function of AHRR is conserved in chicken in terms of the negative regulation of AHR and ERα activities, but its functional mechanism is likely distinct from those of the mammalian and fish homologues.

PS 354 STABLE OVER-EXPRESSION OF PPARβ/δ AND PPARγ TO EXAMINE RECEPTOR SIGNALING IN HUMAN HaCaT KERATINOCYTES.

P. S. Palkar[1], M. G. Borland[1], C. Khozoie[1], B. Zhu[1], C. Lee[1], F. J. Gonzalez[2] and J. M. Peters[1]. *[1]Veterinary Sciences, Pennsylvania State University, State College, PA and [2]Laboratory of Metabolism, National Cancer Institute, Bethesda, MD.*

Recent evidence suggests that peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) can cross-talk with other nuclear receptor signaling, including PPARγ and the retinoic acid receptors (RARs). For example, it was suggested that retinoic acid may promote tumorigenesis by differentially targeting PPARβ/δ rather than RAR, or that PPARβ/δ can repress PPARγ activity and inhibit the chemopreventive properties of PPARγ agonists. To examine PPARβ/δ function and receptor cross-talk in greater detail, the Migr1 retroviral system was utilized to create stable human HaCaT keratinocyte cell lines over-expressing PPARβ/δ and PPARγ. Ligand activation of these receptors in HaCaT keratinocytes over-expressing PPARβ/δ or PPARγ led to enhanced dose-dependent transcriptional upregulation of a known PPAR target gene, angiopoietin-like protein 4 (ANGPTL4) as compared to controls. All-trans retinoic acid (atRA) increased expression of a known RAR target gene in control and HaCaT keratinocytes over-expressing PPARβ/δ. However, despite a high ratio of fatty acid binding protein 5 (FABP5) to cellular retinoic acid binding protein II (CRABP-II), atRA did not increase expression of a PPARβ/δ target gene, even in HaCaT keratinocytes expressing markedly higher levels of PPARβ/δ. Further, while increased efficacy of rosiglitazone to increase ANGPTL4 expression in HaCaT keratinocytes over-expressing PPARγ was observed, over-expression of PPARβ/δ did not modulate the efficacy of rosiglitazone activity in HaCaT keratinocytes as compared to controls. These studies demonstrate that stable over-expression of PPARβ/δ or PPARγ in HaCaT keratinocytes significantly increases the efficacy of ligand activation. Despite overexpression of PPARβ/δ, no changes in retinoic acid-induced, or PPARγ-induced, modulation of target gene expression were observed in HaCaT cells. (Supported by CA124533, CA126826, CA141029, CA140369)

PS 355 PPARβ/δ MODULATES AHR SIGNALING IN MOUSE AND HUMAN KERATINOCYTES.

J. Morales[1], M. G. Borland[1], C. Lee[1], F. J. Gonzalez[2] and J. M. Peters[1]. *[1]Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA and [2]Laboratory of Metabolism, National Cancer Institute, Bethesda, MD.*

Peroxisome proliferator-activated receptors (PPARs) are ligand activated transcription factors that modulate cell-specific biological functions. Previous evidence has shown that PPARβ/δ attenuates tumor promotion and skin carcinogenesis, but PPARβ/δ-dependent modulation of polycyclic aromatic hydrocarbon (PAH) metabolism or tumor initiation has not been specifically examined. The hypothesis that PPARβ/δ modulates aryl hydrocarbon receptor (AHR)-dependent signaling was examined. Surprisingly, *Pparβ/δ*-null primary keratinocytes exhibited reduced PAH-mediated induction of cytochrome P450 (*Cyp*) mRNAs, including *Cyp1a1* and *Cyp1b1*, as compared to wild-type cells. Further functional analyses revealed that *Pparβ/δ*-null keratinocytes exhibit decreased AHR occupancy and histone acetylation at the *Cyp1a1* promoter in response to a PAH. To determine whether this change in occupancy of the *Cyp1a1* promoter was due to alterations in promoter structure, differential DNA methylation was examined in mouse keratinocytes. Bisulfite sequencing of the *Cyp1a1* promoter revealed that *Pparβ/δ*-null keratinocytes had increased DNA methylation, which may explain the lower basal *Cyp* mRNA expression observed in *Pparβ/δ*-null keratinocytes. Moreover, studies using a DNA methylation inhibitor demonstrated that PPARβ/δ modulates AHR signaling, in part, through DNA methylation. Similar modulation of PAH-mediated induction of *Cyp* mRNAs was observed in a human HaCaT keratinocyte cell line stably expressing shRNA against PPARβ/δ. However, inhibition of DNA methylation did not alter PAH-induced expression of *Cyp* mRNAs in this model. Collectively, these findings demonstrate a novel mechanism in mouse and human keratinocytes where PPARβ/δ modulates AHR-dependent signaling. Furthermore, these data suggest that PPARβ/δ may alter the carcinogenic effect of PAHs by modifying the balance between carcinogen bioactivation and clearance. (Supported by CA124533, CA126826, CA141029, CA149369, and the NSF GRFP)

PS 356 ROLE OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN-INDUCIBLE POLY(ADP-RIBOSE) POLYMERASE IN DIOXIN-INDUCED ARYL HYDROCARBON RECEPTOR SIGNALING.

L. M. MacPherson and J. Matthews. *Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). AHR regulates the expression a hundreds of genes including TCDD-inducible poly(ADP-ribose) polymerase (TiPARP, PARP-7). TiPARP is a member of the PARP superfamily, which is an enzyme family that mediates poly(ADP-ribosyl)ation of protein targets. Poly(ADP-ribosyl)ation is post-translational modification associated with a number of biological functions including DNA repair, transcription, apoptosis and cell division. TiPARP contains a C-terminal PARP catalytic domain, a conserved WWE (tryptophan-tryptophan-glutamic acid) domain and a zinc-finger domain. However the biological role of TiPARP and whether TiPARP plays a functional role in AHR signalling is unknown. The aim of the present study was to investigate modulation of TCDD-induced AHR activity by TiPARP. We investigated the effects of loss and overexpression of TiPARP on TCDD-induced cytochrome P450 1A1 (CYP1A1) and CYP1B1 gene expression in T-47D human breast carcinoma and HuH-7 hepatoma cell lines. RNAi-mediated knockdown of TiPARP significantly increased TCDD-induced CYP1A1 and CYP1B1 expression. TiPARP overexpression decreased TCDD-induced CYP1A1- and CYP1B1-regulated reporter activity in a dose-dependent manner. Chromatin immunoprecipitation (ChIP) assays showed increased TCDD-dependent recruitment of AHR to CYP1A1 and CYP1B1 enhancer regions following TiPARP knockdown. TiPARP knockdown also reduced TCDD-induced AHR protein degradation following 24 h treatment. TiPARP truncation mutants revealed TCDD-induced inhibition required the N-terminal (1-234 amino acid residues) as well as the PARP catalytic domain. Single point mutations of two highly conserved residues of the PARP catalytic core motif abolished inhibition of TCDD-induced reporter gene activity. Collectively, these results implicate TiPARP as a negative regulator of AHR signalling.

PS 357 PPARβ/δ INHIBITS VIRAL HRAS1 (V-HRAS1)-INDUCED NEOPLASTIC/MALIGNANT TRANSFORMATION OF MOUSE PRIMARY KERATINOCYTES.

B. Zhu[1], C. Khozoie[1], M. T. Bility[1], N. Blazanin[1], A. B. Glick[1], F. J. Gonzalez[2] and J. M. Peters[1]. *[1]Molecular Toxicology, University Park, PA and [2]Laboratory of Metabolism, National Cancer Institute, Bethesda, MD.*

Ligand activation of PPARβ/δ inhibits chemically-induced skin tumorigenesis. Since Harvey sarcoma ras (Hras1) mutation is a critical event in chemical carcinogenesis, the role of PPARβ/δ in viral Hras1 (v-Hras1)-induced neoplastic transformation was examined in mouse primary keratinocytes. v-Hras1 infected wild-type keratinocytes were protected from malignant transformation, while v-Hras1 infected Pparβ/δ-null keratinocytes underwent malignant transformation. This protection was due in part to the induction of G2/M arrest by ligand activation of PPARβ/δ in wild-type keratinocytes. Flow cytometric analysis revealed that Pparβ/δ-null keratinocytes underwent more active mitosis and adapted to mitotic stress better than wild-type keratinocytes. Further, while ligand activation of PPARβ/δ exerts a negative selection against cells expressing higher levels of the v-Hras1 oncogene by inducing mitosis block in wild-type keratinocytes, Pparβ/δ-null keratinocytes showed a growth advantage in the presence of higher level of v-Hras1 oncogene. To further investigate the mechanism of G2/M induction by ligand activation of PPARβ/δ, microarray analysis was performed. Microarray analysis revealed 62 genes involved in mitosis were induced to a higher level by v-HRAS1 in Pparβ/δ-null keratinocytes as compared to v-Hras1 infected wild-type keratinocytes and ligand activation of PPARβ/δ repressed these changes in wild-type but not Pparβ/δ-null keratinocytes. 33 genes out of these 62 are known to be regulated by the E2F transcription factors. Interestingly, other known E2F regulated genes, notably those involved in G1/S phase of the cell cycle, exhibited a similar pattern of expression as these 62 mitosis-related genes. These results suggest that PPARβ/δ attenuates Hras1-induced neoplastic/malignant transformation by inducing G2/M arrest by interfering with E2F transcription factors in primary keratinocytes. (Supported by CA124533, CA126826, CA141029, CA140369)

PS 358 CHARACTERIZATION OF GENOME-WIDE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-β/δ (PPARβ/δ) BINDING AND TRANSCRIPTIONAL REGULATION.

C. Khozoie[1], M. G. Borland[1], B. Zhu[1], F. J. Gonzalez[2] and J. M. Peters[1]. [1]*Department of Veterinary and Biomedical Sciences and The Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA and* [2]*Laboratory of Metabolism, National Cancer Institute, Bethesda, MD.*

Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ), a member of the nuclear receptor subfamily of transcription factors, modulates gene expression in response to activation by endogenous or exogenous ligands. Whether PPARβ/δ promotes or attenuates carcinogenesis is the subject of much debate. Thus, there is a need to definitively identify and characterize bona fide target genes of this PPAR isoform. Recruitment of PPARβ/δ to specific DNA motifs (PPREs) proximal to target genes results in transcriptional regulation by mechanisms that remain to be fully elucidated. In this study, PPARβ/δ-bound DNA was isolated from C57BL/6 mouse primary keratinocytes by chromatin immunoprecipitation using a highly specific anti- PPARβ/δ antibody developed by this laboratory. Next generation sequencing (ChIP-seq) was performed to map the genomic locations of PPARβ/δ binding (cistrome). This approach was combined with gene expression profiling using cDNA microarrays, enabling the characterization of genome-wide PPARβ/δ cistrome-transcriptome interactions. Novel PPARβ/δ-target genes were identified and confirmed by qPCR and ChIP-qPCR. Results from this analysis indicate that DNA-binding dependent and ligand-dependent transcriptional repression, or activation, together account for over 70% of PPARβ/δ transcriptional regulatory activity. Moreover, results from these studies suggest that differences in DNA binding sequence motifs may directly influence the activity of PPARβ/δ between repression and activation. These findings provide novel insights into the transcriptional regulatory activities of PPARβ/δ that might explain why targeting PPARβ/δ for chemoprevention/chemotherapy is feasible. (Supported by CA124533, CA126826, CA141029, CA140369)

PS 359 FUNCTIONAL CHARACTERIZATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-β/δ (PPARβ/δ) EXPRESSION IN COLON CANCER.

J. E. Foreman[1], W. Chang[2], J. L. Williams[3], M. L. Clapper[2], F. J. Gonzalez[4] and J. M. Peters[1]. [1]*Department of Veterinary and Biomedical Sciences and The Center for Molecular Toxicology and Carcinogenesis, Pennsylvania State University, University Park, PA,* [2]*Cancer Prevention and Control Program, Fox Chase Cancer Center, Philadelphia, PA,* [3]*Cancer Prevention Laboratory, State University of New York at Stony Brook, Stony Brook, NY and* [4]*Laboratory of Metabolism, National Cancer Institute, Bethesda, MD.*

The role of PPARβ/δ in colon carcinogenesis remains uncertain because there are conflicting reports suggesting that this receptor either promotes or attenuates this disease. Previous work suggests that PPARβ/δ is up-regulated by the APC/β-catenin/TCF4 signaling pathway and that the chemopreventive effects of non-steroidal anti-inflammatory drugs (NSAIDs) are mediated in part by repression of PPARβ/δ expression and function. However, these ideas are not supported by all studies. Thus, the present study critically examined the role of PPARβ/δ in human and mouse colon cancer models. Expression of PPARβ/δ mRNA and protein was lower and expression of CYCLIN D1 protein, a verified downstream target of the APC/β-catenin/TCF4 pathway, was higher in human colon adenocarcinomas compared to control tissue. Similar results were observed in colon tumors from $Apc^{+/Min\text{-}FCCC}$ mice compared to control tissue. Dietary administration of sulindac to $Apc^{+/Min\text{-}FCCC}$ mice had no influence on expression of PPARβ/δ in colon or tumors. Stable over-expression of PPARβ/δ in human colon cancer cell lines enhanced ligand activation of PPARβ/δ inhibition of clonogenicity in RKO and HT29 cells. These studies are the most quantitative to date to demonstrate that expression of PPARβ/δ is lower in human colon adenocarcinomas and $Apc^{+/Min\text{-}FCCC}$ mouse colon tumors, consistent with the finding that increasing expression and activation of PPARβ/δ in human colon cancer cell lines inhibits clonogenicity. (Supported by CA124533, CA126826, CA141029, CA140369)

PS 360 REDEFINING ARYL HYDROCARBON RECEPTOR ANTAGONISM THROUGH CHARACTERIZATION OF A NEW LIGAND.

K. J. Smith[1], I. A. Murray[1], R. Tanos[1], Y. Wang[2], J. E. Tellew[2], A. E. Boitano[2], M. P. Cooke[2] and G. H. Perdew[1]. [1]*Pennsylvania State University, University Park, PA and* [2]*Genomics Institute of the Novartis Foundation, San Diego, CA.*

The aryl hydrocarbon receptor (AHR), a ligand activated transcription factor, has been implicated in a variety of physiological functions apart from its classical role in xenobiotic metabolism. Some of these processes involve the AHR interacting with the dioxin response element (DRE), but others are mediated through the AHR's role in the repression of the acute phase response gene *SAA1*. The latter activity led to the classification of a distinct class of ligand, called selective AHR modulators (or SAhRMs) which bind to the AHR but do not elicit the typical DRE-driven response while still suppressing *SAA1*. AHR antagonists have so far been classified only in the context of inhibiting the DRE-driven function of the AHR. In this study, we demonstrate that the compound N-(2-(1H-indol-3-yl)ethyl)-9-isopropyl-2-(5-methylpyridin-3-yl)-9H-purin-6-amine (GNF351) is shown not only to potently antagonize the DRE-driven transcriptional pathway of the AHR, but also to block the repression of *SAA1*, thereby introducing a more complete picture of AHR antagonism. Through a competitive ligand binding assay, GNF351 was shown to bind to the AHR with high affinity. It was demonstrated in both human and murine cells that GNF351 is able to block DRE-driven gene expression in the nanomolar range, through DRE-driven reporter assays and quantitative PCR. GNF351 also does not exhibit partial agonist activity for the AHR at higher concentrations, in contrast to alpha naphthoflavone (αNF). The ability of GNF351 to block the repression of the acute phase response is demonstrated by quantitative PCR upon co-treatment with IL-1β, as well as through a mouse ear edema model upon TPA-induced inflammatory signaling. Therefore, GNF351 offers a more complete and redefined model of AHR antagonism.

PS 361 COMPARISON OF EFFECTS OF DIFFERENT LIGANDS AND Y322 ON ARYL HYDROCARBON RECEPTOR SIGNALING.

M. L. Powis, T. Celius and J. Matthews. *Department of Pharm and Toxicology, University of Toronto, Toronto, ON, Canada.*

The aryl hydrocarbon receptor (AHR) mediates the toxic effects of halogenated aromatic hydrocarbons, but is also activated by a diverse range of compounds. Classic AHR ligands include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and 2,3,7,8-tetrachlorodibenzofuran (TCDF). Other compounds, such as omeprazole (Omp), are thought to activate AHR through phosphorylation of residue Y322. Mutations of the Y322 equivalent in rat AHR prevented activation by Omp but did not effect TCDD activation. In this study, we examined the ability of different compounds to activate AHR-dependent induction of cytochrome P450 1A1 (*CYP1A1*), and hairy and enhancer of split 1 (*HES1*) expression in human breast cancer T-47D and hepatoma HuH7 cells, as well as the role of Y322 in AHR activation by these ligands. Time course mRNA expression analysis showed that in cells treated with TCDD, PeCDF, TCDF or Omp *CYP1A1* mRNA induction increased throughout the 1.5 to 24h treatment, while *HES1* mRNA levels peaked after 1.5h treatment with no increase observed after 24h. Chromatin Immunoprecipitation assays revealed similar recruitment profiles for AHR and coactivator (p300, nuclear receptor coactivator A1/A3) to the regulatory regions of both genes; although some ligand differences were apparent. Proteolytic degradation of AHR was evident after treatment with TCDD, PeCDF, and TCDF, but not with Omp. To investigate the role of Y322 in AHR activation, AHR deficient MCF-7 AHR100 cells were transfected with hAHR, Y322F or Y322A mutants. Y322F resulted in 50% reduction of TCDD- and PeCDF-induced *CYP1A1* mRNA induction and AHR recruitment to *CYP1A1* relative to wt-hAHR, whereas this mutation ablated activation by TCDF or Omp. Y322A mutation failed to induce or recruit AHR to *CYP1A1* following exposure to all compounds. Our findings show that (1) AHR regulates its target genes in temporally distinct manner; (2) different AHR activators recruit similar coactivator following AHR activation; and (3) Y322 is required for maximal ligand dependent activation of AHR.

PS 362 IMMUNOMODULATORY EFFECT OF ARYL HYDROCARBON RECEPTOR ANTAGONISM ALTERS INFLAMMATORY PHENOTYPE IN HFLS-RA CELLS.

T. S. Lahoti, I. A. Murray and G. H. Perdew. *Veterinary Sciences, The Pennsylvania State University, University Park, PA.*

Rheumatoid Arthritis (RA) is a chronic inflammatory disease of unknown etiology which manifests itself, in part, as inflamed human fibroblast-like synoviocytes (HFLS). Upon inflammation, FLS in RA aggressively proliferate to form a pannus, expressing inflammatory mediators such as cytokines, matrix metalloproteinase's (MMPs) and cyclooxygenase 2 (COX2). Epidemiological studies identify a correlation between tobacco smoking and the development and aggressive phenotype of RA. Tobacco smoke is a rich source of agonistic aryl hydrocarbon receptor (AHR) ligands. Thus we hypothesize that inhibition of AHR activity with a potent AHR

antagonist can diminish the inflammatory phenotype of HFLS-RA cells. HFLS-RA cells were pretreated with an antagonist for 1 h prior to 10 ng/ml Interleukin 1B (IL-1B) for 4 h. Inflammatory markers, IL-1B, IL6, COX1 and 2, CCL20, and CD55 were assessed by real time PCR. Cells were also pretreated with an antagonist 1 h followed by IL-1B for an additional 16 h prior to immunoblotting for the expression of COX2 protein. IL-1B treatment significantly enhanced IL-1B, IL6, COX2, CCL20, and CD55 mRNA expression while the AHR antagonist significantly diminished the induced mRNA expression by 2-, 1.3-, 1.8-, 2.1- and 1.7 fold respectively. The levels of COX1 did not alter upon treatment with IL-1B or in combination with antagonist treatment. COX2 mirrored the decrease in mRNA, exhibiting a 1.7 fold decrease in protein expression by AHR antagonism. Identical suppression of IL-1B, IL6, COX2, CCL20 was obtained with the structurally diverse AHR antagonist CH223191 in HFLS-RA cells. These data suggest that AHR antagonism may represent a viable therapeutic target for the amelioration of inflammation associated with rheumatoid arthritis.

PS 363 AH RECEPTOR ANTAGONIST TREATMENT CAN ALTER THE AGGRESSIVE PHENOTYPE OF HEAD AND NECK SQUAMOUS CELL CARCINOMA CELL LINES.

B. DiNatale and G. H. Perdew. *Pennsylvania State University, University Park, PA.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor viewed as binding exogenous ligands and mediating gene transcription. We have previously shown that activated AHR plays a critical role in the induction of progrowth IL6 transcription in numerous tumor cell lines, including head and neck squamous cell carcinoma (HNSCC). HNSCC is an umbrella term for malignancies developing in numerous locations, including the pharynx, larynx, tongue, and oral cavity. These tumors are often aggressive in nature, with high rates of metastasis to regional lymph nodes. Treatment involves surgery to remove lymph nodes and adjuvant chemo- or radiation therapy, although five year survival rates remain below 50%. Our research has shown that there is a level of constitutive activity of the AHR in numerous HNSCC cell lines (e.g. HN13, HN30, HN2095) that have been derived from various locations. This basal AHR activity leads to increased IL6 signaling, which can be repressed by AHR antagonist treatment. This effect is brought about by dismissal of the AHR from upstream DNA of the IL6 promoter. We have shown this DNA binding to be necessary for derepression of the promoter via dismissal of HDAC-containing complexes. In analyzing what effects the constitutive receptor activity could be having aside from IL6 transcription, we have found that AHR activity exacerbates numerous phenotypic aspects that are the hallmark of aggressive HNSCC. AHR antagonist treatment is shown to have an inhibitory effect on proliferation, migration and invasion of multiple HNSCC cell lines, without any cytotoxicity. Additionally, antagonist treatment is able to prevent induction of the drug efflux pump ABCG2/BCRP, pointing to a possible role as a chemosensitizer. For these reasons, AHR antagonist treatment represents a potentially novel adjuvant treatment for HNSCC; one which could aid disease management by inhibiting progression and sensitizing the primary tumor to more aggressive therapies.

PS 364 ROLE OF THE ARYL HYDROCARBON RECEPTOR IN INFLAMMATORY BOWEL DISEASES.

H. Swanson[1], V. Arsenescu[2], E. Choi[1], R. Dingle[1] and R. Arsenescu[3]. *[1]Department of Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY, [2]Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY and [3]Digestive Diseases and Nutrition, University of Kentucky, Lexington, KY.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is best characterized as mediating the adverse effects of agonists such as 2,3,7,8 tetrachlorodibenzo-p-dioxin. Increasing evidence indicates that modification of AHR activity can also impinge on physiological processes and modify pathophysiological processes associated with chronic human diseases. Inflammatory bowel diseases (IBD) are chronic intestinal disorders affecting approximately 1.4 million Americans. The development of IBD requires a genetic predisposition, a dysregulated immune response and undefined "triggers" that appear to include altered lifestyle choices, changes in intestinal colonization and increased industrialization exposures. In this study, we questioned whether alterations in the AHR pathway may modify the severity of IBD by subjecting AHR+/+, AHR+/- and AHR-/- to dextran sulfate sodium-induced colitis. Clinical and morphopathological parameters indicate that lack of the AHR significantly exacerbates disease severity. Examination of AHR protein expression in human biopsy samples indicated that the AHR is expressed primarily within the enterocytes in the healthy controls. In addition, we observed that in biopsies obtained from patients diagnosed with IBD, the AHR was expressed not only within the enterocytes, but also within the lamina propia. Further, gene expression analyses revealed that the mRNA levels of the AHR target gene, CYP1A1 was significantly (approximately 20-fold) upregulated in the IBD patients as compared to the control and correlated with a marker of IBD, IL8 (interleukin 8). These results support the idea that modulation of AHR activity via either the diet, cessation of smoking or pharmacological administration of AHR ligands may be effective strategies with which to treat IBD patients.

PS 365 MULTIPLE INTRACELLULAR SIGNAL MODULATORS AS EFFECTORS OF HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR ACTIVATION.

T. Chen, E. M. Laurenzana and C. J. Omiecinski. *Center for Molecular Toxicology, Penn State University, University Park, PA.*

The human constitutive androstane receptor (hCAR; NR1I3) is an important xenobiotic sensor that modulates the hepatic transcription of ADME-related genes whose products contribute to all three phases of xenobiotic metabolism. Unlike other nuclear receptors, hCAR is maintained in a constitutively active conformation but held quiescent through a tethering mechanism, anchored within a cytosolic protein complex that includes heat shock protein (HSP90). Release and subsequent nuclear translocation of hCAR is triggered through either direct ligand binding, such as CITCO, or indirect acting activators, such as phenobarbital (PB). The molecular mechanisms of hCAR indirect activation remain elusive. In this study we demonstrate that selective inhibitors of protein phosphatase PP2A/PP2B (okadaic acid; OA), P38 MAPK (SB20358), the ubiquitin-proteasome pathway (MG132) and HSP90 (geldanamycin; GA), respectively, all functioned to repress PB-mediated CYP2B6 induction in human primary hepatocytes. However, only OA, GA and MG132 down-regulated the high level of constitutive activity of hCAR in cell-based reporter assays. Interestingly, the latter three modifiers also inhibited CITCO activation of hCAR3, a naturally occurring ligand-dependent hCAR splice variant. Moreover, intracellular localization assays revealed that PB-induced hCAR nuclear trafficking in human primary hepatocytes was completely inhibited by GA, but not by the other three modulators. Mammalian two-hybrid assays further demonstrated that the constitutive interaction of hCAR with the nuclear co-activator, SRC1, was disrupted by OA, GA and MG132, but not SB20358. Taken together, these data suggest that PB-mediated hCAR activation is a complex regulatory scheme, involving multiple intracellular signaling pathways. Further studies are in progress to delineate the specific modalities of receptor function through the dissection of these concerted pathways.

PS 366 CONSTRUCTING A PPARα-MEDIATED TRANSCRIPTIONAL NETWORK IN PRIMARY HUMAN AND RAT HEPATOCYTES.

C. G. Woods[1], K. M. Yarborough[1], L. J. Pluta[1], J. Pi[2], R. S. Thomas[1] and M. E. Andersen[1]. *[1]Computational Biology, The Hamner Institutes for Health Sciences, Research Triangle Park, NC and [2]Translational Biology, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Peroxisome proliferator-activated receptor alpha (PPARα) has been widely targeted for therapeutic treatment of hyperlipidemia, and it is also an inadvertent target of numerous environmental toxicants. In this study, we have evaluated the PPARα gene regulatory network by identifying key transcription factors (TFs) responsible for controlling downstream gene expression and cellular response to PPARα agonist, GW7647. Using TF-DNA binding arrays, activity of over 300 transcription factors was evaluated in human and rat primary hepatocytes. Transcription factors that demonstrated dose-dependent activation were categorized as early, late or sustained based on their temporal response. Early responding TFs with increased activity at 6 hr in human hepatocytes included factors binding to the Ras-responsive transcription element (RSRFC4), several liver specific factors, including one with sequence homology to hepatocytes nuclear factors 3 (HNF3) and a TF with sequence homology to acute inflammatory factor serum amyloid A (SAA). Both HNF3 and SAA were also activated early and sustained to 72 h, along with several STAT proteins and related interferon-gamma activated factors. Factors that were activated as early as 6 hr and sustained to 72 hr included AP-2/Sp1, cMyb and NF-κB, which are involved in cell growth. Despite the fact that gel-shift assays show dose-dependent activation of PPARα as early as 6 hr and dose-dependent increase in expression of target genes CYP4A11, ACOX1 and HMGCS2 is observed by 12 hr, the TF-DNA arrays only showed increased activation of PPARα and RXR/DR-1 across all doses at 72 hr. Comparative experiments using rat hepatocytes and

RNAi were also performed to identify TFs required for peroxisomal biogenesis. Ultimately, the findings from this study and additional results from gene arrays will be used to construct a circuit diagram that describes PPARα-mediated signaling and predicted cellular outcomes.

PS 367 MODE OF ACTION (MOA) AND DOSE-RESPONSE APPROACHES FOR NUCLEAR RECEPTORS.

D. Schrenk[5], R. Budinsky[1], J. Corton[3], C. Elcombe[2], J. Klaunig[4] and D. Wolf[3]. *[1]Dow, Midland, MI, [2]CRX Biosciences, Dundee, Scotland, United Kingdom, [3]U.S. EPA, Research Triangle Park, NC, [4]Indiana University, Indianapolis, IN and [5]University of Kaiserslautern, Kaiserslautern, Germany.*

Sub-threshold doses for non-cancer and (in appropriate cases) cancer has been the dominant paradigm in risk assessment, but the application of dose-response modeling approaches with a threshold have been questioned in a 2009 NRC report. A recent workshop at the NIEHS explored the development of dose-response approaches to nuclear receptor-mediated liver cancer, within a Mode of Action (MOA) Human Relevance Framework (HRF). Case studies addressed activation of AHR, CAR, and PPARalpha receptors. For each case study a diverse and multi-disciplinary panel of experts from academia, industry, government and other organizations evaluated the key events leading to tumors. Each panel addressed whether the biology of the nuclear receptor necessitates a minimum threshold of ligand to affect activation, gene expression, and subsequent biological and toxicological responses. The panels also briefly discussed whether linear low-dose modeling was appropriate, based on the underlying science of nuclear receptor signaling biology. The MOA/HRF provided a weight-of-evidence approach for evaluating these charge questions against the available data. The AHR expert panel, for the first time in an expert panel format, rigorously applied the MOA/HRF mode of action framework and agreed on a mode of action. Similarly, the CAR expert panel identified relevant data and applied the framework with emphasis on the qualitative and quantitative aspects of human relevance. For PPARalpha, the expert panel built upon previous applications of the framework using significant new data that allowed for refinement of the key event descriptions and updated considerations related to human relevance. Each panel identified key data needs and suggested improvements for application of the MOA/HRF framework. The public workshop had broad support and funding from industry, government, universities, scientific societies, and research organizations. This abstract does not necessarily reflect EPA policy.

PS 368 TESTING STRUCTURE-BINDING PREDICTIONS FROM COMPARATIVE MODELING OF THE AHR LIGAND-BINDING DOMAIN: STUDIES WITH ZEBRAFISH AHR1a.

S. I. Karchner[1], D. G. Franks[1], A. Pandini[3], A. A. Soshilov[2], L. Bonati[3], M. S. Denison[2] and M. E. Hahn[1]. *[1]Woods Hole Oceanographic Institution, Woods Hole, MA, [2]University of California, Davis, CA and [3]Universita' degli Studi di Milano-Bicocca, Milan, Italy.*

The aryl hydrocarbon receptor (AHR) is required for the toxic and biochemical effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Studies of AHR diversity in vertebrate species reveal dramatic differences in AHR function among and within species, including among multiple AHR paralogs in fish and birds. Differences in AHR function contribute to species and strain differences in sensitivity to TCDD, but the structural basis for such differences in function is not well understood. Comparative modeling of AHR structure can generate predictions that can be tested experimentally through targeted mutagenesis and functional assays. We performed comparative homology modeling of ligand-binding (PAS-B) domains of the three zebrafish AHR paralogs, one of which (AHR1a) is unable to bind TCDD and is transcriptionally inactive. Analysis of the binding cavity revealed reduced volume of AHR1a (295 Å3) as compared to AHR1b (632 Å3) and AHR2 (675 Å3), both of which exhibit high-affinity binding of TCDD. Comparative modeling identified three residues predicted to contribute to the small binding pocket of AHR1a. Site-directed mutagenesis of each residue alone and in all combinations revealed that the Y296H and T386A mutations restored the ability of AHR1a to bind [3H]TCDD and form TCDD- and ARNT-dependent protein-DNA complexes. The Y296H / T386A double mutant exhibited a synergistic increase in function. Together, these results demonstrate the value of an approach combining comparative modeling with experimental testing to understand the structural basis of AHR function and the role of AHRs in species differences in sensitivity to TCDD. [Supported by grants R01ES006272, R01ES007685, and R01ES012498.]

PS 369 DISCOVERING TRANSCRIPTIONAL REGULATORS OF CYTOCHROME P450 1A THROUGH SIRNA HIGH-THROUGHPUT SCREENINGS.

P. Solaimani[1], O. Hankinson[1] and R. Damoiseaux[2]. *[1]Molecular Toxicology IDP, University of California Los Angeles, Los Angeles, CA and [2]California NanoSystems Institute, University of California Los Angeles, Los Angeles, CA.*

Using RNAi high throughput technologies, we have set out to identify proteins that modulate the Aryl Hydrocarbon Receptor (AhR)-dependent induction of the CYP1A1 gene in the Hepa-1 murine hepatic cancer cell line. Dioxins such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and certain polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene, are environmental contaminants and potent carcinogens. The mechanism of their toxicities lies in the ligand mediated binding to AhR and subsequent activation of its target genes. The resulting metabolism of PAHs by the target gene CYP1A1 plays a major role in carcinogenesis, namely through the formation of electrophilic derivatives that can mutate DNA. In addition to its role in carcinogenesis, AhR has been shown to have a plethora of physiological roles and understanding AhR-dependent induction of CYP1A1 can lead to insight into other AhR mediated genes. Further characterization of the mechanism(s) of AhR dependent induction of gene transcription is therefore a very important research objective. An siRNA library targeted against 6000 proteins in the druggable genome has been used to transfect Hepa-1 cells, which were treated with TCDD for 24 hours, and then assayed for CYP1A1 activity using the EROD assay. Following RSA statistical analysis we identified 90 hits with a p-value of 0.005 or less that are potentially involved in CYP1A1 transcriptional regulation. To confirm these hits as true positives, we will use endoribonuclease prepared siRNAs as a cost effective means to follow up on all our top hits in the screening. Using this high throughput method we expect to identify novel proteins involved in dioxin induced CYP1A1 expression.

PS 370 EPITHELIAL REGULATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ BY CHEMICAL RIBOSOME-INACTIVATING STRESS.

S. Park, H. Choi, H. Yang and Y. Moon. *Department of Microbiology and Immunology, Pusan National University School of Medicine and Medical Research Institute, Yangsan, Republic of Korea.*

Based on our recent study that peroxisome proliferator-activated receptor gamma (PPARγ is a negative regulator of mRNA stability of pro-inflammatory cytokine, PPARγ was linked to a notable mRNA stabilizing protein, HuR in presence of ribosome-inactivating stress. CCAAT-enhancer-binding protein (C/EBP) homologous protein (CHOP) is a crucial stress-responsive factor in various mucosal injuries including ribosome-inactivating stress. In the present study, chemical ribosome-inactivating stresses were assessed for their effects on stress-inducible CHOP expression and its association with epithelial inflammatory cytokine production. Several representative ribotoxic agents enhanced CHOP expression and its nuclear translocation in human intestinal epithelial cells. Whereas CHOP was a strong positive regulator of cytokine production, IL-8 production was inversely associated with expression of the mucosal regulatory factor, PPARγ. Expression of exogenous PPARγ suppressed ribotoxin-triggered cytoplasmic translocation of HuR. In contrast, PPARγ-regulating CHOP was a positive modulator of HuR protein export from nuclei. Taken together, the results indicate that ribotoxin-induced CHOP protein is positively associated with production of pro-inflammatory cytokine IL-8, but down-regulates PPARγ action, subsequently allowing the cytosolic translocation of HuR protein and stabilization of IL-8 mRNA in gut epithelial cells (This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2009-0065479)).

PS 371 FOXA1 IS ESSENTIAL FOR THE AHR-DEPENDENT REGULATION OF CYCLIN G2.

S. Ahmed, S. Al-Saigh and J. Matthews. *Pharmacology & Toxicology, University of Toronto, Toronto, ON, Canada.*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor known to mediate the toxic and carcinogenic effects of a wide variety of environmental contaminants such as dioxin (TCDD; 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin). AHR is thought to play a protective role in breast cancer development by blocking cell proliferation. This might occur through direct interaction with estrogen receptor alpha (ERα) or indirectly through modulation of ERα target genes. One such gene of interest is *cyclin G2* (*CCNG2*) which inhibits cell cycle progression by preventing cell cycle entry. This gene is negatively regulated by ERα, which might contribute to estrogen-dependent tumor growth. Our laboratory has shown

that *CCNG2* is positively regulated by TCDD via AHR activation, suggesting that AHR might utilize this pathway to suppress tumor growth. However, the mechanism by which AHR regulates *CCNG2* is unknown. Using chromatin immunoprecipitation in T-47D human breast cancer cells we report that the prototypical AHR ligand TCDD (10nM) is able to recruit AHR and nuclear coactivator 3 (NCoA3) to the enhancer region of *CCNG2* (60% and 10% compared to a 5% total) leading to a 2-fold increase in gene expression after 6h of treatment with TCDD(10nM). Furthermore, our data reveal that the transcription factor Forkhead box A1 (FOXA1) is recruited to *CCNG2* in a TCDD-dependent manner. FOXA1 is known to be an important pioneer factor controlling the binding of ERα to its target genes. RNAi-mediated knockdown of FOXA1 abolished TCDD-dependent recruitment of AHR to *CCNG2* and reduced *CCNG2* expression levels. Interestingly, knockdown of FOXA1 also caused a marked decrease in ERα, but not AHR protein levels. However, RNAi-mediated knockdown of ERα had no effect on TCDD-dependent AHR recruitment to or expression of *CCNG2*. Our data show that FOXA1, but not ERα is essential for AHR-dependent regulation of *CCNG2*, assigning a role for FOXA1 in AHR action.

PS 372 DIVERGENT ROLES FOR THE ARYL HYDROCARBON RECEPTOR AND ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR IN ESTROGEN SIGNALING.

M. P. Labrecque[1], K. J. Tam[1], B. D. Hollingshead[2], G. G. Prefontaine[1], G. H. Perdew[2] and T. V. Beischlag[1]. [1]*Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada and* [2]*Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA.*

The aryl hydrocarbon receptor (AHR) and the aryl hydrocarbon receptor nuclear translocator (ARNT) form a heterodimeric transcription factor upon binding a variety of environmental contaminants, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The activated AHR/ARNT complex (AHRC) regulates the expression of xenobiotic target genes to combat the effects of environmental contaminants and evidence has shown that TCDD represses estrogen receptor (ER) target gene activation through the AHRC. Our data indicates that AHR and ARNT may act independently of each other at off-target sites so we set out to determine the specific functions of AHR and ARNT in estrogen dependent signalling. Human MCF7 breast cancer cells and human ECC-1 endometrial carcinoma cells were transfected with short inhibitory RNA's (siRNA) to AHR or ARNT. Cells were treated with vehicle, 17β-estradiol (10nM), TCDD (2nM) or a combination of ligands for 16 h and target gene expression and protein levels were assayed by real-time PCR and Western blotting, respectively. Knockdown of AHR with siRNA abrogates dioxin-inducible repression of estrogen-dependent gene transcription. Intriguingly, knockdown of ARNT does not effect TCDD-mediated repression of estrogen-regulated transcription. Furthermore, basal and activated levels of cathepsin-D and progesterone receptor mRNA are downregulated in MCF7 cells but upregulated in ECC-1 cells in response to loss of ARNT. These responses are mirrored at the protein level with cathepsin-D but progesterone receptor seems to be refractory. We have obtained experimental evidence demonstrating a dioxin-dependent repressor function for AHR and a dioxin-independent coactivator/corepressor function for ARNT in estrogen signalling. These results provide us with further insight into the mechanisms of transcription factor crosstalk and putative therapeutic targets in estrogen positive cancers.

PS 373 THE ARYL HYDROCARBON RECEPTOR INTERACTS WITH ATP5α1, A SUBUNIT OF THE ATP SYNTHASE COMPLEX, AND MODULATES MITOCHONDRIAL FUNCTION.

D. M. Tappenden[1, 2], S. Lynn[1, 2], R. B. Crawford[3], N. E. Kamniski[2, 3], R. S. Thomas[4] and J. J. LaPres[1, 2]. [1]*Biochemistry, Michigan State University, East Lansing, MI,* [2]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI,* [3]*Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI and* [4]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Polyaromatic hydrocarbons (PAHs), such as dioxins are prevalent environmental contaminates produced by both natural and man-made activities. 2,3,7,8 tetrachloro-dibenzo-p-dioxin (TCDD) is the canonical molecule in this family of compounds. The aryl hydrocarbon receptor (AHR), a ligand activated transcription factor and a member of the PAS super-family of environmental sensors, mediates cellular responses to PAHs. A complete understanding of the signaling pathways involved in the toxicity induced by ligand activated AHR has not been elucidated. To explore the potential influences of protein-protein interactions in AHR-mediated toxicity, a tandem affinity purification (TAP) and mass spectrometry strategy was utilized to isolate and identify proteins that co-purify with the AHR. The known protein interactions between the AHR, Hsp90, and ARA9 and the ligand-dependent interaction with ARNT were confirmed, demonstrating the validity of the approach. Using this method a novel interaction between the AHR and ATP5α1, a subunit of the ATP synthase complex, has been established. This interaction was confirmed by co-immunoprecipitation in wild type cells. The ATP synthase complex is localized to the inner membrane of the mitochondria and is essential for cellular energetics. Investigation of the interaction between the AHR and ATP5α1 has revealed that a fraction of the total cellular content of the AHR localizes to the mitochondria. Furthermore, exposure to TCDD leads to hyperpolarization of the mitochondrial inner membrane, in an AHR dependent manner, while cellular ATP levels are maintained. These findings support a role for the AHR in mitochondrial function and cellular energy homeostasis that is disrupted in TCDD exposure.

PS 374 REGULATION OF CHOLESTEROL BIOSYNTHETIC GENE EXPRESSION BY AH RECEPTOR THROUGH A DNA-INDEPENDENT MECHANISM.

R. Tanos, R. D. Patel and G. H. Perdew. *Penn State University, University Park, PA.*

The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor. Activation of AHR by xenobiotic agonists such as TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is known to have toxic consequences through driving the expression of target genes, namely CYP1A1, by binding to dioxin response element sequences in their promoter region. Interestingly, Ahr-null mice exhibit a defined set of physiological phenotypes that indicate an endogenous role for AHR. Recent microarray studies performed in mice and rats treated with TCDD have shown a decrease in the expression of lipid metabolism genes. In our effort to understand the mechanisms of this regulation, we conducted a microarray analysis on liver samples from ligand-treated transgenic mice expressing a DNA-binding mutant AHR (A78D) on an Ahr-null background and showed that AHR DNA-binding is not essential for suppression of genes involved in cholesterol synthesis. To alleviate the phenotype of Ahr-null mice, we established a mouse line expressing a liver specific DNA-binding mutant AHR on a CreAlb/AhrFx/Fx background. Real-time qPCR performed on these mice as well as wild-type mice confirmed repression of cholesterol biosynthesis genes namely HMGCR, SQLE and LSS following receptor activation in both mouse lines. Conversely, using real-time qPCR and western blot analysis, we showed that absence of the receptor in liver-specific CreAlb/AhrFx/Fx mice leads to upregulation of those genes in the absence of an exogenous ligand. These data firmly establish the role of AHR as regulator of cholesterol biosynthesis independently of its DNA-binding ability and suggest that AHR may be a previously unrecognized therapeutic target.

PS 375 MULTI-SPECIES ANALYSES OF DIRECT ACTIVATORS OF THE CONSTITUTIVE ANDROSTANE RECEPTOR.

C. Omiecinski[1], D. M. Coslo[1], T. Chen[1] and R. C. Peffer[2]. [1]*Center for Molecular Toxicology, Penn State University, University Park, PA and* [2]*Syngenta Crop Protection, Inc., Greensboro, NC.*

The constitutive androstane receptor (CAR; NR1I3) is a member of the superfamily of nuclear receptors that functions as an important xenochemical sensor in mammalian cells. Upon chemical activation, CAR undergoes nuclear translocation and heterodimerization with the retinoid X receptor to subsequently interact with DNA targets to transcriptionally modulate gene expression. CAR is unusual among nuclear receptors in that it possesses a high level of constitutive activity in cell based assays, obscuring the detection of direct ligand activators. However a human splice variant of CAR, termed hCAR3, exists that exhibits negligible constitutive activity; rather, CAR3 is activated by ligands with similar specificity as the reference form of the receptor, CAR1. In this study, we hypothesized that similar hCAR3 variant receptors could be constructed across various mammalian species' forms of CAR, thereby preserving species specific ligand responses yet enabling their detection in a much more sensitive ligand-dependent mode. Therefore, in this study, a battery of CAR3 variant receptors were produced for the respective native CARs of mouse, rat and dog, and comparatively evaluated with selected ligands in mammalian cell reporter assays. The results demonstrate that the 5 amino acid insertion that typifies human CAR3 also creates ligand activated receptor function in other species' CARs, while maintaining signature responses in each species to select CAR ligands. These variant constructs permit evaluation of differential effector chemical responses across species and facilitate, for example, comparative study of tumor promotional properties of suspected hepatocarcinogens on direct CAR activation responses in different species.

PS 376 A FUNCTIONAL CROSSTALK BETWEEN CAR AND LXR LINKS XENOBIOTIC RESPONSE AND LIPOGENESIS.

W. Xie, T. Wada and Y. Zhai. *University of Pittsburgh, Pittsburgh, PA.*

The liver X receptor (LXR) and constitutive androstane receptor (CAR) are two nuclear receptors postulated to have distinct functions. LXR is a sterol sensor that promotes lipogenesis, whereas CAR is a xenosensor that controls xenobiotic responses. Here we show that LXRα and CAR are functionally related in vivo. Loss of CAR increased the expression of lipogenic LXR target genes, leading to increased hepatic triglyceride accumulation; whereas activation of CAR inhibited the expression of LXR target genes and LXR ligand-induced lipogenesis. Conversely, a combined loss of LXR α and β increased the basal expression of xenobiotic CAR target genes; whereas activation of LXR inhibited the expression of CAR target genes and sensitized mice to xenobiotic toxicants. The mutual suppression between LXRα and CAR was also observed in cell culture and reporter gene assays. LXRα, like CAR, exhibited constitutive activity in the absence of an exogenously added ligand by recruiting nuclear receptor co-activators. Interestingly, although CAR competed with LXRα for co-activators, the constitutive activity and recruitment of co-activators was not required for CAR to suppress the activity of LXRα. In vivo chromatin immunoprecipitation (ChIP) assay showed that co-treatment of a CAR agonist compromised the LXR agonist responisve recruitpment of LXRα to Srebp-1c, whereas a LXR agonist inhibited the CAR agonist responisve recruitment of CAR to Cyp2b10. In conclusion, our results have revealed dual functions of LXRα and CAR in lipogenesis and xenobiotic responses, establishing a unique role of these two receptors in integrating xenobiotic and endobiotic homeostasis.

PS 377 SPECIES-DEPENDENT AND RECEPTOR-SELECTIVE EFFECT OF BILOBALIDE ON THE ACTIVITY OF CONSTITUTIVE ANDROSTANE RECEPTOR AND PREGNANE X RECEPTOR.

A. Lau[1], G. Yang[1], G. Rajaraman[2], C. C. Baucom[3] and T. K. Chang[1]. [1]*Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC, Canada,* [2]*CellzDirect - Life Technologies, Austin, TX and* [3]*CellzDirect - Life Technologies, Durham, NC.*

Bilobalide is a sesquiterpene present in Ginkgo biloba. This naturally-occurring chemical was reported to increase CYP2B, but not CYP3A, gene expression in rat hepatocytes. However, the molecular basis of this differential effect is not known. CYP2B and CYP3A expression are regulated by constitutive androstane receptor (CAR; NR1I3) and pregnane X receptor (PXR; NR1I2), which control the transcription of genes involved in diverse biological functions, including bioactivation and detoxification of toxicants. Therefore, we determined whether bilobalide differentially modulates rat CAR (rCAR) and rat PXR (rPXR) activities. Given the pronounced species differences in chemical activation of CAR and PXR, we also determined its effect on human CAR (hCAR) and human PXR (hPXR) activities. HepG2 human hepatoma cells and cultured rat hepatocytes were transfected with a reporter plasmid, an internal control plasmid (Renilla luciferase), and a rCAR, hCAR, rPXR, or hPXR expression plasmid. Bilobalide activated rCAR, as assessed by a luciferase reporter gene assay, and it increased rCAR target gene expression in rat hepatocytes, as analyzed by CYP2B1 mRNA and catalytic activity assays. In contrast, it did not activate rPXR, hCAR, or hPXR in reporter gene assays. Consistent with these results, bilobalide did not alter rPXR, hCAR, or hPXR target gene expression in rat or human hepatocytes, as evaluated by CYP3A23, CYP2B6, and CYP3A4 mRNA and catalytic activity assays. Additional analysis indicated that bilobalide did not attenuate rPXR-, hCAR-, or hPXR-mediated reporter gene activity, suggesting that it was not a ligand of these receptors. In conclusion, bilobalide is a species-dependent and receptor-selective activator of rCAR, and it may be useful as a toxicological tool to delineate the role of CAR in chemically-induced toxicity in rat models.

PS 378 THE HUMAN XENORECEPTOR CAR AND ITS NATURALLY-OCCURRING SPLICE VARIANTS DISPLAY DIFFERENTIAL PROTEIN-PROTEIN INTERACTIONS WITH RXRα AND SRC1.

E. M. Laurenzana and C. J. Omiecinski. *Center for Molecular Toxicology and Carcinogenesis, Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA.*

Modulation of gene transcriptional activities by nuclear receptors involves a complex interplay of numerous proteins that have diverse functions. The human constitutive androstane receptor (hCAR or hCAR1; NR1I3) and two of its splice variants, CAR2 and CAR3, dimerize with the retinoid X receptor alpha (RXRα) to effect their transcriptional modulation. Further, nuclear co-activators, including steroid receptor co-activator 1 (SRC1), also interact with the CAR-RXRα dimer to form an active receptor complex. Previous studies have indicated that CAR1 does not require ligand binding for interaction with RXRα, however it is unclear whether ligand is required for CAR2/ or CAR3/RXRα heterodomerization. To investigate the mechanisms of CAR activation and heterodomerizaton, we conducted mammalian two-hybrid assays in COS-1 cells to assess the interaction of CAR variants, RXRα and SRC1 in the presence and absence of the pan-CAR ligand, CITCO. In cells co-transfected with CAR1 and RXRα, robust levels of reporter gene expression were detected in both the presence and absence of CITCO, suggesting that CAR1 interacts readily with RXRα, even in the absence of ligand. In contrast, the interaction of CAR2 and CAR3 with RXRα was dependent on ligand. However, additional studies demonstrated that CAR1's interaction with SCR1 was both ligand and RXRα independent, whereas CAR2 requires both ligand and RXRα for interaction with SRC1. Further, SRC1 enhances CAR2 interaction with RXRα. Interestingly, CAR3 required ligand for its interaction with SRC1, but not RXRα, while the presence of SRC1 enhanced the CAR3/RXRα interaction. The results of these studies suggest that CAR1 and its splice variants, CAR2 and CAR3, interact differentially with both receptor ligands and with nuclear co-regulators and that these differential interactions likely mediate distinct gene regulatory activities contributed by each of the hCAR variants.

PS 379 EFFECTS OF TRICLOSAN ON CELL PROLIFERATION AND PPARα ACTIVATION IN MOUSE AND HUMAN CELLS.

Q. Wu, F. A. Beland and J. Fang. *Division of Biochemical Toxicology, National Center for Toxicological Research/FDA, Jefferson, AR.*

Triclosan is a widely used antimicrobial agent found in consumer products such as antibacterial soaps, toothpaste, and cosmetics. In an 18-month mouse oral carcinogenicity study, triclosan induced a significant increase in liver neoplasms that was attributed to the activation of peroxisome proliferator-activated receptor α (PPARα). In this study, we have compared the effects of triclosan on cell proliferation and PPARα activation in mouse and human cells. Mouse Hepa-1c1c7, NIH-3T3, and human HepG2 and THLE2 cells were treated with 0.5, 2.5, 5, 10, or 20 μM triclosan and changes in cell growth, necrosis, apoptosis, cell cycle progression were determined. In addition, the effects of triclosan on mouse and human PPARα were investigated using nuclear receptor reporter assays. Triclosan caused a dose-dependent inhibition of cell growth, increased cell necrosis and apoptosis, particularly at the highest concentrations, but had no effect upon cell cycle progression. When using a mouse PPARα receptor reporter assay, triclosan increased the PPARα activities significantly at concentrations ≥ 5 μM. In contrast, triclosan showed antagonistic activities in a human PPARα receptor reporter assay at the two highest concentrations, both in the presence and absence of 20 nM GW590735, a PPARα receptor agonist. These data indicated that triclosan has similar effects upon cell proliferation in transformed mouse and human cell lines but show differential activity towards mouse and human PPARα. (Supported by Interagency Agreement 224-07-0007 between NCTR/FDA and NIEHS/NTP.)

PS 380 GLOBAL PANCREATIC GENE EXPRESSION ANALYSIS OF FISHER-344 MALE RATS INDICATES SLC2A4 (GLUCOSE TRANSPORTER) GENE TO PLAY IMPORTANT ROLE IN THE TOXICITY EFFECTS OF 2-AMINOANTHRACENE EXPOSURE.

W. E. Gato and J. C. Means. *Chemistry & Biochemistry, Southern Illinois University-Carbondale, Carbondale, IL.*

The effect of 2-aminoanthracene (2-AA) on the islets of Langerhans was investigated by exposing twenty four post-weaning 3-4 week old F-344 male rats to 0 mg/kg (control), 50 mg/kg (low dose), 75 mg/kg (medium dose) and 100 mg/kg (high dose) 2-AA for 14 and 28 days followed by analysis of the pancreas for broad gene expression changes by Affymetrix Microarray GeneChips. Arylamine 2-AA is an aromatic hydrocarbon employed in the manufacturing of agricultural chemicals, drugs, dyes, road tars, synthetic fuels, inks, plastics as rubber antioxidants, curing agents in the synthesis of epoxy resins and of polyurethanes. Other sources of exposure of this chemical include tobacco smoking and cooked foods. Results showed more gene ontology (GO) categories were found to be significant for the two week study than the four week study. With respect to the 14 day study, 45, 57 and 237 cellular component, molecular function and biological process gene ontology categories were found to be significant respectively. Similarly, a respective 7, 5 and 25 cellular component, molecular function and biological process. Some of these GO categories include; modification-dependent protein catabolic process, phospholipid biosynthetic process, glucose metabolic process, small ribosomal subunit, ubiquitin

ligase complex, proteasome complex and Golgi apparatus, ligase activity, cofactor binding and sequence-specific DNA binding. To explore the relationships between differentially expressed genes, VisANT, interactive software platform that enables biological network modeling was employed to analyze the dataset. Three mRNA transcripts were identified from the network plot to have at least 50 links with other genes. These include SLC2A4 (glucose transporting gene), MAPK3 (a signal transduction pathway protein, mitogen activated protein kinase 3 and RAD23B (DNA repair protein and ubiquitin-like containing protein).

PS 381 EFFECTS OF RIFAMPIN ON DRUG METABOLIZING ENZYME AND TRANSPORTER GENE EXPRESSION IN HUMAN HEPATOCYTES.

Q. Yang, U. Doshi and A. P. Li. *in vitro ADMET Laboratories, Advanced Pharmaceutical Sciences, Columbia, MD.*

Clinically significant drug-drug interactions can occur by induction or inhibition of not only drug metabolizing enzymes, but also the uptake and efflux transporters. We report here a qPCR assay for the quantification of gene expression of P450 isoforms, phase II enzymes (UGTs; SULTs), and both uptake (OATPs; OCTPs) and efflux (MDRs; MRPs) transporters and the results of this assay with the model P450 inducer, rifampin. Plateable cryopreserved human hepatocytes were plated in 24-well plates at a cell density of approximately 0.35 million cells/well. The cells were overlaid with Matrigel at approximately 4 hours after plating. After 2 days in culture, the cells were treated for 3 days with solvent control or rifampin at concentrations of 1 to 20 uM. Under this culturing condition, the human hepatocytes exhibited epithelial cell morphology and were near 100% confluent. Results showed that rifampin treatment on human primary hepatocytes resulted in a dose-dependent induction of CYP3A4 gene expression, with a maximum induction of over 10X of that for solvent control. The treatment also caused statistically significant induction of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP3A5 and CYP3A7 and UGT1A1. No apparent effects were observed for CYP1A1, CYP2D6, CYP2E1 and CYP4A11. Statistically significant alterations on several drug transporters, such as MDR2, MRPs, and OCT1 were observed. Our results show that treatment of human primary hepatocytes with rifampin led to alterations of the gene expression of a variety of drug metabolizing enzymes and transporters, with CYP3A4 induction being the most extensive. As rifampin is a known PXR ligand, the results confirm the current belief that PXR is involved in the regulation of a large number of genes, including P450, UGT, SULT and various transporter genes. The gene expression results suggest that rifampin-induced drug interactions may involve not only CYP3A4, but also non-CYP3A4 drug metabolizing enzyme and transporters.

PS 382 MICROTUBULE ACETYLATION ENHANCES BINDING AND INTRACELLULAR TRAFFICKING OF PLASMID DNA IN GENE TRANSFER.

M. A. Badding and D. A. Dean. *Pediatrics, University of Rochester Medical Center, Rochester, NY.* Sponsor: B. Lawerence.

For gene delivery to be successful, the genetic material must cross several obstacles as it moves through the cell to the nucleus for gene expression, making this approach less than optimal. One of the barriers is the cytoskeletal meshwork within the cytoplasm, but our lab and others have demonstrated that the microtubule network is actually required for plasmid movement to the nucleus. We have shown that there is a subset of modified microtubules that remain intact even after cytoskeletal disruption by drugs or mechanical strain and are post-translationally acetylated. Experiments in which microtubule acetylation is increased by inhibition of the tubulin deacetylase HDAC6 show greater transfection efficiency and more rapid localization of DNA to the nucleus. Based on these findings, we hypothesize that acetylated microtubules bind more DNA through adapter proteins than do non-acetylated microtubules, resulting in more rapid movement of DNA to the nucleus. To test this, two variations of an in vitro microtubule-binding spin-down assay were employed, using purified tubulin, cell extract, and plasmid DNA. First, real time PCR was used to quantify pelleted DNA with either unmodified or acetylated microtubules. We found that more DNA pelleted with acetylated microtubules than with unacetylated microtubules. Next, immunofluorescence was used to visualize the co-localization of both microtubule-binding motor proteins and plasmid DNA to unmodified or acetylated microtubules. We found that motor proteins and DNA co-localize with acetylated microtubules more so than with unacetylated microtubules. Current studies are underway to evaluate movement in real-time with live cell microinjection studies. We hypothesize that plasmids will have greater rates of movement in cells with highly acetylated microtubules compared to those with fewer modifications. Taken together, these findings will provide a foundation for determining how modulation of microtubule acetylation can be used as a means to increase intracellular trafficking of plasmid DNA and enhance gene therapy.

PS 383 INHIBITION OF A549 LUNG CANCER CELL MIGRATION BY PRECURSOR LET-7G MICRORNA.

S. Park, A. Minai Tehrani and M. Cho. *Laboratory of Toxicology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea.*

Let-7g microRNAs, short non-coding RNAs 21 nucleotides long, suppress protein translation by binding to the 3'of target messenger RNAs. Deficient expression of let-7g is related with the poor prognosis of lung cancer patients. Compared to normal lung cells, let-7g expression is Deficient in lung cancer cells (NSCLC). Moreover, K-Ras and HMGA2 are well known as targets of let-7g. In this study, we assessed the potential role of precursor let-7g in lung cancer cell metastasis, focusing on the two targets of let-7g, HMGA2 and K-Ras. We showed that pre-let-7g repressed the migration of A549 lung cancer cells via HMGA2-mediated E2F1 down-regulation. Therefore, our results suggest that pre-let-7g could be used as a target for the inhibition of lung cancer cell migration.

PS 384 ELUCIDATING THE CONSTITUTIVE ACTIVITY OF THE HUMAN TNIP1 PROMOTER.

P. C. Encarnacao, C. Zhang and B. J. Aneskievich. *Pharmacology/Toxicology, University of Connecticut, Storrs, CT.*

Our laboratory isolated a novel nuclear receptor interacting protein, TNFα induced protein 3 interacting protein 1 (TNIP1), and characterized it as a corepressor of retinoic acid receptors (RARs) and peroxisome proliferator-activated receptors (PPARs). RARs and PPARs are pharmacologic targets for control of various pathologies including, but not limited to diabetes, psoriasis, photoaging, and various cancers. As TNIP1 can repress activity of RARs and PPARs, it becomes crucial to understand what controls its expression levels. We isolated ~6kb of the human TNIP1 promoter and examined it both in silico and experimentally for transcriptional control elements. Sequence analysis by MatInspector predicted two specificity protein (Sp) sites in the proximal region of the promoter. We hypothesized the Sp family of transcription factors would contribute to TNIP1 promoter's constitutive activity. Transcriptional activation studies revealed TNIP1 is positively regulated by Sp1 and Sp3. Furthermore, EMSA and chromatin immunoprecipitation demonstrated the physical association between Sp1 and Sp3 and specific regions of TNIP1 promoter. Decreased Sp1 protein via siRNA or Sp binding to cognate sites by mithramycin decreased TNIP1 mRNA. In summary, we have demonstrated that Sp1 and Sp3 contribute to the constitutive regulation of TNIP1 promoter through two sites proximal to the transcription start site. Changes in endogenous Sp levels or pharmacological control of its activity would be expected to affect TNIP1 expression, which, in turn, could ultimately regulate RAR and PPAR activity.

PS 385 GESTATIONAL *UDP-GLUCURONOSYLTRANSFERASE 1A* GENE REGULATION IS CONTROLLED BY THE PREGNANE X RECEPTOR.

S. Chen and R. H. Tukey. *Pharmacology, University of California, San Diego, La Jolla, CA.*

Chen S and Tukey RH. Laboratory of Environmental Toxicology, Departments of Chemistry & Biochemistry and Pharmacology, University of California, San Diego, La Jolla, CA 92093.
UDP-glucuronosyltransferases (UGTs) detoxify endogenous substances originating from normal metabolic and catabolic processes. Clinical observations indicate that UGT1A proteins in women during pregnancy are induced as a gestational adaptation to facilitate the amplified metabolic burden. We have shown using transgenic *UGT1* mice (Chen S, et al., JBC 280:37547, 2005) the human-specific gestational upregulation of the *UGT1A* genes. In current studies, the *UGT1* locus has been placed into a murine *Ugt1*$^{-/-}$ background, creating a humanized *UGT1* (*hUGT1*) mouse model (Fujiwara R, et al., PNAS 107:5024, 2010). Gestational upregulation of hepatic *UGT1A* expression in pregnant *hUGT1* mice was initiated at gestation day (GD) 14.5, followed by a drastic increase on GD16.5, implying gene regulation is driven primarily by pregnancy hormones peaking during late gestation. Primary hepatocytes isolated from *hUGT1* mice were screened with estrogens, progestins, and glucocorticoids. Corticosterone, a natural form of rodent glucocorticoid, significantly induced *UGT1A1* expression. Similar results were observed with the synthetic glucocorticoid dexamethasone (DEX). Potential glucocorticoid involvement extended our interests into the transcription factors pregnane X receptor (PXR) and constitutive androstane receptor (CAR). Humanized *UGT1* mice were bred into a murine *Pxr*$^{-/-}$ or murine *Car*$^{-/-}$ background, creating *hUGT1/Pxr*$^{-/-}$ and *hUGT1/Car*$^{-/-}$ mice. Gestational upregulation of the *UGT1* locus was conserved in *hUGT1/Car*$^{-/-}$ mice, but completely absent in *hUGT1/Pxr*$^{-/-}$ mice. Similarly, both *hUGT1* and *hUGT1/Car*$^{-/-}$ mice responded to DEX treatment with

UGT1A gene induction, while this response was lost in *hUGT1/Pxr*$^{-/-}$ mice. In conclusion, PXR mediates the gestational upregulation of the human *UGT1A* genes, which may be driven by elevated glucocorticoid levels during late gestation. (Supported by USPHS Grant GM086713 and ES010337)

PS 386 BREAST MILK SUPPRESSES *UDP-GLUCURONOSYLTRANSFERASE 1A1* GENE EXPRESSION IN THE GASTROINTESTINAL TRACT AND INCREASES THE RISK FOR SEVERE HYPERBILIRUBINEMIA AND BRAIN DAMAGE.

R. Fujiwara, S. Chen and R. H. Tukey. *Pharmacology, University of California, San Diego, La Jolla, CA.*

Premature and term newborns are susceptible to physiological jaundice, which can lead to acute bilirubin encephalopathy or the more permanent clinical sequelae of bilirubin-induced neurological dysfunction (BIND). Accumulating evidence indicates that breast-fed infants have a higher risk for developing BIND than formula-fed infants. However, the underlying mechanism linking severe hyperbilirubinemia to breast feeding has not yet been elucidated. Here we describe a new mechanism that places breast-fed neonates at risk for developing BIND. Humanized *UDP-glucuronosyltransferase 1* (*hUGT1*) mice (Fujiwara R, et al. PNAS 107:5024-9, 2010) develop severe hyperbilirubinemia during neonatal development. The steady-state levels of total serum bilirubin (TSB) during development are concordant with expression of UGT1A1 in the gastrointestinal tract. Approximately 10% of neonatal *hUGT1* mice develop BIND, characterized by seizures and the deposition of bilirubin in the brain. In contrast, formula-fed *hUGT1* mice have significantly lower levels of TSB and do not develop seizures. Compared to breast-fed neonates, formula-fed *hUGT1* mice have significantly induced levels of UGT1A1 in the GI tract. Formula feeding has no effect on *UGT1A1* gene expression in liver. The induction of UGT1A1 by formula may be controlled by xenobiotic receptors PXR and CAR, since the *Cyp3a11* and *Cyp2b10* genes are also induced. This data indicates that GI tract control of *UGT1A1* gene expression in newborns is sufficient in preventing developmentally induced hyperbilirubinemia. As we define the role of breast milk in regulating *UGT1A1* gene expression, *hUGT1* mice may prove useful as a screening tool in examining nutritional supplements to aid in halting the progression of hyperbilirubinemia. (Supported by USPHS grants GM086713 and ES010337)

PS 387 TRANSCRIPTOMIC ANALYSIS REVEALS MECHANISM UNDERLYING THE OFF-TARGET EFFECT OF THE CHOLESTERYL-ESTER TRANSFER PROTEIN INHIBITOR TORCETRAPIB.

J. Hoflack, N. Flint, M. Haiker, A. Stauffer, L. Suter-Dick, A. Roth, T. Weiser, T. Singer, L. Müller, E. J. Niesor and R. G. Clerc. *F Hoffmann La Roche, Basel, Switzerland.*

The development of the cholesteryl-ester transfer protein (CETP) inhibitor Torcetrapib failed in a phase III clinical study. An effect on the aldosterone pathway was suggested as responsible for the increased mortality. We therefore evaluated the impact of Torcetrapib and of Angiotensin II on the transcriptome of the human aldosterone-producing adrenocortical cell line H295R using microarrays and qRT-PCR. Several key genes involved in adrenal-dependent steroidogenesis were robustly regulated by Torcetrapib at concentrations as low as 1 nM. Torcetrapib essentially elicited a transcriptomic pattern similar to that of Angiotensin II on the aldosteroidogenesis pathway, but displayed also distinct features. In particular, upregulation of the L-type Ca2+ channel alpha1c mRNA by Torcetrapib at the top of the pathway, which was not observed with Angiotensin II, characterizes the different properties of the two aldosteroidogenic agents. Consistently, inhibition of Torcetrapib-dependent aldosteroidogenesis by the L-type-specific Ca2+ channel inhibitor Nifedipine, not of the Angiotensin II-dependent aldosteroidogenesis, as well as inhibition of aldosteroidogenic gene regulations occuring in response to Torcetrapib, underscored the specific contribution of L-type Ca2+ channels in the signaling cascade of Torcetrapib. This Torcetrapib-specific effect occurs at the mRNA level to a wide extent and unravels a new activation of the signaling cascade towards aldosterone production, through L-type Ca2+ channels. Dalcetrapib, a CETP-targeting compound from a chemical class different from Torcetrapib, had no observable effect on either aldosterone production nor on aldosteroidogenic gene regulations up to 10 μM under identical experimental conditions. These transcriptome data, confirmed later using various functional endpoints, first indicated that the Torcetrapib-effect is not an on-target but a chemical class-specific, off-target effect.

PS 388 TRANSCRIPTIONAL INDUCTION OF GLUTATHION S-TRANSFERASE BY CHLORPYRIFOS IN HEPG2 CELLS.

I. M. Medina[1], M. C. Martinez[1], M. Rubio[1], A. E. Rojas[1], M. L. Robledo[1], M. I. Giron[1] and G. Elizondo[2]. [1]*Secretaria de Investigacion y Posgrado, Universidad Autonoma de Nayarit, Tepic, Nayarit, Mexico and* [2]*bDepartamento de Biología Celular, Centro de Investigación y de Estudios Avanzados del IPN, Mexico, DF, Mexico.* Sponsor: B. Quintanilla.

Organophosphates pesticides (OPs), such as chlorpyrifos, are the most widely used insecticides worldwide. As a result of their extensive use, large populations of humans are potentially expose to low doses of OPs, mainly due to the presence of these compounds in food and drinking water. Glutathion S-transferase (GST) catalyzes the nucleophilic attack of the tripeptide glutathione (GSH) on electrophilic substrates, thus forming an important line of defense and protecting cell components from reactive molecules. The action of GST on organophosphate pesticides can lead to activation or detoxification. The aim of this study was to test the hypothesis that OPs pesticides (chlorpyrifos) modulate the expression of GST alpha (GSTA) and theta (GSTT) gene in HepG2 cells. To determine the expression of GSTA and GSTT, quantitative real-time PCR assay (rtPCR) of the transcripts was performed with gene-specific fluorescent labeled probes and the activity assays was determined using the GST Assay Kit. Treatment with chlorpyrifos resulted in an induction of GSTT and GSTA mRNA and activity relative to a control (untreated cell cultures). These findings demonstrate that these insecticides modulate transcription of GSTT and GSTA enzymes and may play an important role in the modulation of endogenous compounds and xenobiotic metabolism. Studies are in progress to define the molecular mechanisms by which chlorpyrifos induce the expression of GSTT and GSTA.

PS 389 IDENTIFICATION OF TRANSCRIPTIONAL REGULATORS OF C. ELEGANS METALLOTHIONEIN GENE EXPRESSION.

J. Hall and J. H. Freedman. *NIEHS, Durham, NC.*

The carcinogenic metal cadmium can induce various intracellular stresses. Analysis of transcriptome data from multiple species indicate that cadmium exposure alters the expression of hundreds of genes that are regulated by multiple signal transduction pathways, many of which remain to be defined. In response to cadmium, cells increase the expression of highly conserved, small, cysteine-rich metal-binding proteins known as metallothioneins (MTs), which function in metal detoxification. The nematode *C. elegans* has two MT genes: mtl-1 and mtl-2. To identify regulatory factors and pathways that control metal-inducible mtl-1 transcription, integrated transgenic strains of *C. elegans* containing GFP under the control of the 5'-regulatory region of mtl-1 were constructed; pmtl-1::GFP. Transgenic strains constitutively express GFP in the pharynx and following cadmium exposure, express GFP in the intestine. Using the pmtl-1::GFP strains, genes involved in various stress response pathways were tested for their potential role in controlling mtl-1 expression. Knockout of akt-1 or akt-2 did not affect GFP expression; however, the knockout of both genes simultaneously increased expression. AKT-1 is a serine/threonine kinase involved in the insulin signaling pathway and complexes with AKT-2 to regulate transcription of downstream factors. PDK-1 directly interacts with this complex and the knockout of pdk-1 resulted in an increase in expression. Interestingly, mtl-1 transcription was not affected when other insulin signaling pathway genes were knocked out. This suggests that PDK-1 and the AKT-1/2 complex act independently of this pathway to control mtl-1 transcription. To identify other transcriptional regulators, transcription factors involved in various MAPK pathways were tested. Knockout of atf-7 which is involved in the JNK/p38 pathway resulted in an increase in GFP expression. Pathway analysis and RT-PCR data indicate that ATF-7 regulates cadmium-inducible MT transcription downstream of PDK-1 and AKT-1/2 and that this regulation is independent of the insulin signaling pathway.

PS 390 ANILINE-INDUCED CELL CYCLE PROGRESSION OF SPLENOCYTES: RESPONSE OF CYCLINS AND CYCLIN-DEPENDENT KINASES IN G1/S AND G2/M PHASES.

J. Wang, G. Wang and M. Khan. *University of Texas Medical Branch, Galveston, TX.*

Aniline exposure is associated with toxicity to the spleen leading to splenomegaly, hyperplasia, fibrosis, and a variety of sarcomas of the spleen on chronic exposure in rats. In earlier studies, we have shown that aniline exposure leads to iron overload, oxidative stress and activation of redox-sensitive transcription factors, which could regulate various genes leading to a tumorigenic response in the spleen. We have also shown that aniline exposure leads to enhanced expression of cyclins and cyclin-dependent kinases (CDKs) in G1 phase of cell cycle. However, molecular mechanisms

leading to aniline-induced cell cycle progression beyond G1 phase in the spleen remain unknown. This study was, therefore, undertaken on the regulation of G1/S and G2/M phase cell cycle proteins and their mRNA levels, and expression of CDKs in the spleen, in an experimental condition preceding a tumorigenic response. Male SD rats were treated with aniline (0.5 mmol/kg/day via drinking water) for 30 days (controls received drinking water only), and protein expression of cyclins and CDKs and their mRNA levels were measured. Splenocytes from aniline-treated rats showed significantly increased protein expression of cyclins E, A, cyclin B along with decreased expression of p27, as compared to the controls. Similarly, real-time PCR analysis showed significantly increased mRNA expression for cyclin E, A, cyclin B and decreased expression of p27 in the spleens of aniline-treated rats. The overexpression of the cyclins was associated with increases in the expression of CDK2 and CDC2 as well as phosphorylation of CDK2 and CDC2 proteins. Our data suggest that increased expression of cyclins, CDKs and phosphorylation of CDK2 and CDC2 proteins could contribute to aniline-induced tumorigenic response in the spleen. Supported by NIH ES06476.

PS 391 ENHANCED PDE4B EXPRESSION AUGMENTS LPS-INDUCIBLE TNF-α IN GLUCOSE-PRIMED MONOCYTES: A POTENTIAL PATHOGENIC ROLE FOR PHOSPHODIESTERASES (PDES) IN THE DEVELOPMENT OF DIABETIC COMPLICATIONS.

E. Chambers[1], L. Gobejishvili[1], S. Joshi-Barve[1], C. McClain[1, 2] and S. Barve[1]. *[1]Department of Medicine/GI, University of Louisville, Louisville, KY and [2]Robley Rex Veterans Administration Medical Center, Louisville, KY.*

Diabetes mellitus (DM) is a chronic disease that has no cure. Research shows that inflammation plays a pivotal role in the development of diabetes; DM is associated with an enhanced inflammatory response and a propensity to develop multi-organ dysfunction. Diabetics experience increased gut permeability, and therefore LPS-inducible cytokines are highly relevant in this disease. Elevated levels of pro-inflammatory cytokines, such as TNFα, IL-8, IL-6 and advanced glycation end products (AGEs) together play a major role in the evolution of diabetic complications. Our earlier work demonstrated that cAMP and monocyte/macrophage-specific PDE4B play a key role in the LPS-inducible expression of pro-inflammatory cytokines in monocytes. Our recent observations demonstrate that human monocytes exposed to high glucose (HG–15 mM) exhibit a "primed phenotype", wherein they express elevated levels of these pro-inflammatory cytokines (TNFα, RAGE {Receptor for AGE}, IL-8 and IL-6) in response to LPS stimulation. We hypothesize that HG exposure results in enhanced PDE4 expression, thereby decreasing intracellular cAMP concentrations, and enhancing TNFα expression following LPS stimulation. Accordingly, in this study we examined the involvement of cAMP/PDE4B in the high glucose mediated enhancement of TNFα following LPS stimulation. We found that in comparison with normal glucose (NG–5.5 mM); HG significantly enhances the expression of LPS-inducible TNFα at both the mRNA and protein levels. Importantly, HG in combination with LPS, was observed to enhance PDE4B expression indicating its involvement in the up-regulation of pro-inflammatory cytokine expression via down-regulation of cellular cAMP levels. These data strongly implicate PDEs in the pathogenic dysregulation of pro-inflammatory cytokines suggesting that PDEs are a potential therapeutic target for diabetic patients.

PS 392 CURCUMIN EPIGENETICALLY REACTIVATES SILENCED TUMOR SUPPRESSOR GENE - TISSUE FACTOR PATHWAY INHIBITOR-2 AND DECREASES CELL SURVIVAL AND INVASIVENESS IN HEPATOCELLULAR CARCINOMA CELLS.

A. Moghe[1], A. S. Barve[1], D. Barker[1], S. Ghare[1], L. Gobejishvili[1], S. Joshi-Barve[1], C. McClain[1, 2] and S. Barve[1]. *[1]Department of Medicine/GI, University of Louisville, Louisville, KY and [2]Robley Rex Veterans Administration Medical Center, Louisville, KY.*

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer-related mortality. In HCC, epigenetic modifications involving histone deacetylation and aberrant promoter methylation are implicated in the inactivation of tumor suppressor (TS) genes, which have a significant impact on carcinogenesis. Tissue factor pathway inhibitor-2 (TFPI-2), a Kunitz-type serine protease inhibitor, is a tumor suppressor gene that is epigenetically silenced in approximately 90% of all HCCs and most HCC cell lines. Restoration of TFPI-2 expression in tumor tissue has been shown not only to inhibit invasion, tumor growth, metastasis and angiogenesis but also to induce apoptosis. Curcumin, a phenol, was investigated for its effects on TFPI-2 expression and HCC cell survival using HepG2 cells. We observed significant cell death when HepG2 cells were exposed to curcumin. Corresponding to the cell death, curcumin robustly reactivated TFPI-2 expression in these cells. We examined the histone modifications and DNA methylation status at the TFPI-2 promoter using the chromatin immunoprecipitation and bisulfite conversion techniques, respectively. Our studies revealed that curcumin increases histone H3 acetylation at the promoter. Minimal DNA methylation was observed at the TFPI-2 promoter and curcumin did not affect this methylation status. These data suggest histone acetylation plays a dominant role in the reactivation of TFPI-2. Curcumin is able to re-establish the transcriptional competence of the silenced TFPI-2 gene in HCC cells by reversing the repressive heterochromatic state via histone H3 acetylation. Curcumin-mediated reactivation of TFPI-2 gene expression was also associated with reduced cell migration. These data suggest that curcumin is a potential anti-tumor agent in the prevention and therapy of HCC.

PS 393 THE HEPATOCYTE AUTONOMOUS CLOCK MODULATES THE CHRONOTOXICITY OF ACETAMINOPHEN.

B. P. Johnson[1], J. Walisser[1], Y. Liu[1], A. Shen[1], E. McDearmon[2], B. McIntosh[2], A. Schook[2], J. Takahashi[2] and C. Bradfield[1]. *[1]Oncology, University of Wisconsin-Madison, Madison, WI and [2]Northwestern University, Evanston, IL.*

The hepatotoxicity of Acetaminophen (APAP) was first reported in the 1960s and its circadian changes in metabolism in the 1970s. The dose independent circadian variation in APAP hepatotoxicity is thought to be primarily due to oscillations of liver GSH and possibly changes in cytochrome p450 enzymatic activity which follow eating patterns. The circadian clock drives the 24 hour oscillations in these factors directly and indirectly (e.g. by direct regulation of drug metabolizing enzymes or by driving feeding rhythms). Because the circadian clocks of various tissues can become uncoupled or disrupted under conditions such as shift work, we set out to determine the relative contributions of the central clock in the suprachiasmatic nucleus (SCN) and the hepatocyte circadian clock in modulating the chronotoxicity of APAP. Using mice with conditional null alleles of the Mop3 locus, we were able to generate livers harboring hepatocytes with little or no cell autonomous circadian rhythms. The observation that these mice become resistant to the chronotoxicity of APAP, under the same feeding and activity rhythms, suggests that while the central clock modulates detoxification indirectly through feeding rhythms and GSH; the hepatocyte clock controls daily oscillations in major aspects of APAP bioactivation by the cytochrome P450 system.

PS 394 ARSENITE EXPOSURE DOWN-REGULATES GLUTAMATE TRANSPORTER EXPRESSION IN GLIAL CELLS.

Y. Castro-Coronel[1, 2], A. Ortega[1], L. Del Razo[2] and E. Lopez-Bayghen[1]. *[1]Department of Genetica y Biol.Mol., CINVESTAV, México, Mexico and [2]Department . Toxicología, CINVESTAV, México, Mexico.*

Chronic exposure to inorganic arsenic (iAs) damages the CNS. Glutamate (Glu), the major excitatory amino acid, has proven to be highly neurotoxic when its levels in the synaptic cleft are not tightly regulated. A family of Na^+-dependent excitatory amino acid transporters (EAATs) clear Glu from the synaptic space. Within the cerebellum, the activity of the Bergmann glial transporter EAAT1/GLAST, accounts for more than 90% of Glu uptake. Since iAs exposure results in toxic insults, we explored here if EAAT1/GLAST constitutes a molecular target for this metalloid. To this end, primary cultures of chick cerebellar Bergmann glial cells were exposed to sodium arsenite for 24 h and EAAT1/GLAST activity was recorded via [^{3}H]-D-aspartate uptake. A decrease in Glu transport was found through a PKA, PKC and $p38^{MAPK}$-dependent diminution in V_{max} without an apparent change in K_M. To rule out a decrease in cell viability, this parameter was determined via MTT assays with no cell death associated to the metalloid exposure. To gain insight into the molecular mechanisms of arsenite action on EAAT1/GLAST activity, generation of ROS or lipoperoxidative damage were investigated. Neither an increase in ROS nor a clear augmentation of lipid peroxidation, were present. Nevertheless, an antioxidant response was recorded as an increase in Nrf2 DNA binding and a rise in GSH levels. Long-term iAs exposure resulted in *chglast* transcriptional down-regulation. A sharp decrease in *chglast* mRNA levels (qRT-PCR) and promoter activity (reporter assays) suggested that EAAT1/GLAST is in fact, a molecular target of iAs. (Funded by Conacyt 50414 Mexico).

PS 395 DOWN-REGULATION OF TWO HSP FAMILIES BY TNIP1 IN HACAT KERATINOCYTES.

V. P. Ramirez, C. Zhang, W. Krueger and B. J. Aneskievich. *Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT.*

Increased levels of TNFα induced protein 3 interacting protein 1 (TNIP1) mRNA is detected in inflammatory diseases such as psoriasis and rheumatoid arthritis. Nevertheless, TNIP1 reduces NF-κB activation, and represses nuclear receptor ac-

tivity, specifically peroxisome proliferator activated receptors and retinoic acid receptors. To better understand increased TNIP1 possibly contributing to such disease states, we over-expressed recombinant TNIP1 protein in HaCaT keratinocytes and performed a microarray analysis to determine pathways and possible target genes. At a cutoff of a 2-fold change or greater, elevated TNIP1 resulted in 94 significantly down-regulated genes and 3 up-regulated genes. Pathway analysis determined inflammatory response, cell death, and cellular growth/proliferation as the top network functions associated with the dataset. Increased TNIP1 significantly decreased mRNA levels of several genes encoding for two heat shock protein (HSP) families, HSPA (HSP70) and DNAJ (HSP40). The top ten down-regulated genes included HSPA6 (HSP70B') and DNAJB1 (HSP40), being downregulated 20-fold and 5-fold, respectively. Heat shock proteins play integral roles not only protecting cells in times of stress, but are also vital in tissue homeostasis. These results indicate a role for TNIP1 as a novel regulator of heat shock protein expression; therefore TNIP1 may play a role in regulation of cellular stress and the pathogenesis of inflammatory diseases.

PS 396 SEXUALLY DIMORPHIC ROLE FOR THE CIRCADIAN CLOCK IN MODULATING XENOBIOTIC METABOLISM.

L. A. Hooven, K. Sherman, E. Chow, L. Beaver and J. Giebultowicz. *Oregon State University, Corvallis, OR.*

The circadian clock synchronizes physiological and molecular rhythms with predictable daily oscillations in the environment such as light and temperature. There are multiple examples of sexual dimorphism in circadian output. Although many xenobiotic metabolizing genes are expressed in a coordinated daily rhythm, sexual dimorphism in clock modulation of these genes has been little investigated. Here we examine how the circadian clock differentially modulates gene expression and response to pesticides in males and females, using both wild type flies and flies deficient in the core clock genes CYC and PER. Flies were maintained in 12h:12h light/dark and collected for qRT-PCR or enzyme activity, or acutely exposed to a series of doses of propoxur or fipronil for one hour, every four hours for 24 hours. In wild type flies, cytochrome P450 enzyme activity exhibited dissimilar daily rhythms in males and females, as did 24h gene expression profiles of Cyp6g1, Cyp6a8, Cyp4e2, and the nuclear receptor HNF4. The significant daily rhythm in LC50 we previously reported in male flies in response to propoxur was absent in females, which exhibited a much greater LC50 across the day. The response to fipronil showed a similar rhythm in male and females. A mutation in CYC results in increased resistance to propoxur in male flies, but not females. This mutation also results in increased female resistance to fipronil, with no change in male response. Conversely, a mutation in PER, part of the negative arm of the clock, results in decreased resistance to propoxur in males and in females. The same mutation causes no change in the male response to fipronil, but decreased resistance in females. We are currently comparing daily expression profiles in nuclear receptors and xenobiotic metabolizing genes in CYC and PER mutant flies in order to examine sex-specific clock regulation of these genes. Importantly, this work reveals that disruption of the circadian clock, an important environmental sensor, differentially affects the ability of males and females to respond to toxicants.

PS 397 CHARACTERIZATION OF THE PROMOTER-PROXIMAL REGION OF HUMAN MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 4 GENE.

J. E. Manautou and X. Gu. *Pharmaceutical Sciences, University of Connecticut, Storrs, CT.*

Multidrug resistance-associated protein 4 (MRP4, ABCC4) is an efflux transporter localized in the basolateral membrane of hepatocytes. Hepatic MRP4 expression is very low and highly variable. MRP4/Mrp4 is inducible in mice and human by toxic APAP exposure. Activation of transcription factors Nfe2l2, PPARα and CAR mediate Mrp4 induction under a variety of conditions. However, knowledge on MRP4/Mrp4 promoter structure and function is very limited. Our previous work showed that transcription activity of human MRP4 promoter region is constitutively active and that the activity of the promoter-proximal region is controlled by many activating (e.g., NRF1, SP2, STAT1, TFAP2A) and repressive (e.g., HES1, KLF15, ZFP161) transcription factors. In this study, we further characterized the promoter-proximal region of MRP4 gene. Alignment of human and mouse 5' regulatory sequence shows 60%-70% homology in the promoter-proximal region and much less homology in the distal region. Increased transcription activity of reporter gene is only detected when the upstream promoter DNA sequence extends beyond 152 bp relative to MRP4 coding sequence starting site. Furthermore, over-expression of E2F transcription factor 1(E2F1) in HepG2 cells up-regulates, while transcription factors POZ (BTB) and AT hook containing zinc finger 1(PATZ1) and ELK1 (member of ETS oncogene family) down-regulates MRP4 reporter gene expression for constructs containing upstream DNA fragments from 5 bp to 212bp relative to MRP4 coding sequence starting site. Over-expression of E2F1, which plays a crucial role in controlling cell cycle and as a tumor suppressor protein, resulted in much higher induction of MRP4 promoter reporter activity (>10 fold) than other transcription factors tested. These results suggested that MRP4 gene expression might be coupled and tightly regulated by cell cycle. The core promoter for MRP4 gene and physical interaction between these cis-elements and transcription factors in liver will be further identified or characterized. This work was supported by The National Institutes of Health Grant DK069557.

PS 398 HIGH CONTENT ANALYSIS USING REDISTRIBUTION® TECHNOLOGY.

A. M. Peters, D. Miller, B. Samson and Y. Fedorov. *Life Science Research - Cellomics, Thermo Fisher Scientific, Pittsburgh, PA.* Sponsor: J. Haskins.

Understanding transcriptional activity and how it interacts with various stimuli can gain insight to cellular processes and instabilities that cause toxic outcomes such as cell stress or death in a range of conditions. In this study, four different regulators of transcriptional activity (HIF-1α, ATF6, Rad51 and p53) were monitored using the Redistribution technology. With this technology, proteins are GFP-tagged and observed for accumulation and translocation of GFP chimera within the cell after treatment. This approach enables visualization of cellular processes in a more natural state, without having to add multiple stains, permeabilization steps, and correct antibodies. Frozen cells were plated into 96-well plates, treated, fixed, Hoechst-stained, and run on the Cellomics® ToxInsight™ IVT platform. Information about individual cell morphology, cellular toxicity, and various GFP intensity outputs were observed. For Hypoxia Inducing Factor 1 alpha (HIF-1α), unstimulated cells exhibit a low protein level and thus show little GFP; however, upon treatment with a compound that mimics hypoxia (2,2'-dipyridyl), HIF-1α accumulates in the cell, producing larger levels of GFP. Activator of Transcription factor 6 (ATF6) was observed to translocate from the cytoplasm/ER to the nucleus after treatment with tunicamycin. Treatment with compounds inducing DNA double-stranded breaks lead to accumulation of the Rad51-GFP chimera in the nuclear foci. Both interaction of p53 and Hdm2 and p53 translocation can be visually observed when treated with various compounds: Nutlin-3 caused increased fluorescence in the nucleus (blocking p53-Hdm2 interaction), while forskolin caused non-specific localization of GFP chimera in the cytoplasm. By using Redistribution technology and high-content imaging analysis, we were able to not only look at overall intensity accumulation (which can be done using other methods), but could, on a cell-by-cell basis, focus on localization, translocation between sites, as well as morphological changes within the cell at one time.

PS 399 ASSESSMENT OF NUCLEAR FACTOR KAPPA B (NF-κB) SIGNALING IN THE HIPPOCAMPUS DURING KANIC ACID EXPOSURE USING TRANSGENIC REPORTER MICE.

J. A. Miller[1], R. A. Bialecki[2] and R. B. Tjalkens[1]. [1]*Center for Environmental Medicine, Colorado State University, Fort Collins, CO and* [2]*Safety Assessment U.S., AstraZeneca, Wilmington, DE.*

NF-κB is a key transcriptional regulator of numerous genes including those involved in regulating cell death / survival pathways and cellular plasticity. Chemical induced kindling of seizure activity results from sustained alterations in neuronal excitability, indicative of neuronal plasticity resulting in hyper-excitability. In the present study, we employed a unique transgenic reporter mouse expressing NF-κB dependent GFP, in order to investigate the role of NF-κB signaling in rendering hippocampal neurons hyper-excitable. Mice were treated with kainic acid (KA, 2 x 10 mg/Kg, ip) over 48 hr and NF-κB activation was assessed 24 hours following treatment. Animals displayed mild behavioral changes consistent with the early stages of seizure induction including generalized immobility and facial clonus which progressed to include mild head nodding after the second dose of KA. Assessment of reporter expression showed that under basal conditions GFP is absent in the hippocampus except for a pronounced expression in the CA3 region pyramidal layer. After KA treatment, increased expression of GFP in the CA3 region and marked expression in the stratum moleculare, dentate gyrus molecular layer and in the dentate hilus were observed. These preliminary data show selective regional effects of KA on NF-κB activation which mimic the sensitivity of these regions to KA induced excitoxicity. This demonstrates the potential utility of this reporter model in investigating NF-κB activation in seizure induction and maintenance and may also prove useful in detecting chemically-induced effects on the hippocampus that may underly seizuregenic activity of pharmaceutical compounds.

PS 400 ALTERATIONS OF SIGNALING PROTEIN INTERACTIONS IN RESPONSE TO INCREASING DOSE.

C. Kinzer, H. Williams, X. Gao, N. Rubenstein, A. Cook, J. Vrana and J. Boyd. *Bennett Department of Chemistry, West Virginia University, Morgantown, WV.*

Although current understanding of signal transduction within biological networks is based upon changes in protein phosphorylation over time, cells are also responsive to increasing doses of inhibitors independent of time. By assessing the functional response of a cellular network to increasing doses of inhibitors, additional information can be gained about the relationships and roles of proteins in response to cellular stress. To determine the direction of information flow in a cellular network in response to stress, the phosphorylation activity of several protein kinases within the mitogen-activated protein kinase (MAPK) pathways were monitored. HepG2 cells were exposed to increasing doses of deguelin, and KCN. Correlation and protein activity were quantified using multiplex phosphoprotein assays on lysates obtained 400 minutes after dosing. The resulting correlation analysis across all dosing intervals was in general agreement with accepted temporal models of signal transduction. However, correlation values compared on a dose-to-dose level differed significantly from the temporal model and revealed surprising changes in specificity among protein kinases. This preliminary analysis into interactions of signaling proteins in response to increasing dose offers a unique way of framing cellular network relationships in biochemical networks, and holds the potential to aid in the eventual prediction of toxicity by identifying cellular strategies for adaptation and survival.

PS 401 IMPACT OF ENERGY METABOLISM ON THE DESIGN OF KINASE-TARGETED THERAPEUTICS.

A. Cook, H. Williams, X. Gao and J. Boyd. *Bennett Department of Chemistry, West Virginia University, Morgantown, WV.*

Protein kinases play key roles in signaling pathways that regulate cellular functions such as proliferation and apoptosis. Due to the potential regulatory control over such important biological processes, interest in kinase-targeted chemotherapeutics has grown exponentially in the drug discovery arena. Current drug design is aimed at developing substrate-competitive kinase inhibitors, which are thought to offer higher selectivity and sensitivity over ATP-competitive kinase inhibitors that must compete with intracellular ATP concentrations. However, what has not been explored as comprehensively is the impact that these inhibitors can have on energy metabolism. This study seeks to investigate the activity of two different AKT and GSK3 inhibitors, substrate-competitive versus an ATP-competitive, in relation to glycolysis and oxygen consumption. Our results suggest that beyond the effects of inhibiting metabolically-linked proteins, ATP-competitive inhibitors may alter intracellular interpretation of available ATP which leads to decreases in compensatory energy metabolism and could result in greater toxicity.

PS 402 cAMP-DEPENDENT PATHWAY(S) INCREASE P27^{KIP}-CYCLIN D1 THROUGH THE RAP-GTP/B-RAF MAPK SIGNALING PATHWAY IN RENAL CANCER.

N. Mastrandrea, K. Y. Tham, J. D. Cohen, T. J. Monks and S. S. Lau. *Pharmacology/Toxicology, University of Arizona, Tucson, AZ.*

The Eker rat (Tsc-2EK/+) bears a germline mutation in the tuberous sclerosis-2 (Tsc-2) tumor-suppressor gene, and can undergo spontaneous and chemically induced loss of heterozygosity. Renal tumors null for tuberin, the Tsc-2 gene product, derived from 2,3,5-tris-(glutathion-S-yl)hydroquinone (TGHQ) treated Tsc-2EK/+ rats, display elevated p27 and cyclin D1 levels. Similar elevations were observed in TGHQ transformed QTRRE renal epithelial cells derived from Tsc-2EK/+ rats, which also exhibit high ERK, B-Raf and Raf-1 kinase activities. p27 signaling pathways, especially those associated with its binding partner, cyclin D1, are relatively unknown. In QTRRE cells cAMP signaling regulates both p27 expression and Rap-GTP/B-Raf activation of the MAP kinase cascade. We now report that dibutyryl cAMP (db-cAMP; 1mM) or the phosphodiesterase inhibitor, theophylline (0.6 mg/ml), produced a time-dependent increase in p27 protein levels (1.4, 2.0, 3.0 fold, and 1.6, 2.8, 2.4 fold at 2, 10 and 24 hrs, respectively). A concomitant increase in cyclin D1 levels was also observed in db-cAMP and theophylline treated QTRRE cells. Inhibition of Raf kinases, with either sorafenib (50 μM) or B-Raf siRNA resulted in MAPK down-regulation of p27. Moreover, following a 48 hr treatment of QTRRE cells with p27 siRNA there was an equivalent 88% decrease in both p27 and cyclin D1 protein levels; similarly, a 48 hr treatment with cyclin D1 siRNA resulted in a concomitant 50% decrease in p27 protein levels, suggesting that the p27-cyclin D1 complex promotes preservation of both proteins. Collectively these data reveal that the cAMP/Rap1b/B-Raf pathway modulates the expression of p27 and cyclin D1 in Tsc-2 gene regulated-renal cancer. Therefore, the loss of tuberin and engagement of the cAMP pathway may independently direct p27-cyclin D1 stabilization during renal tumor formation. (P30ES006694, T32ES007091, AstraZeneca Studentship)

PS 403 EFFECT OF P, P'-DDE ON JAK2, STAT1α, AND NFκB ACTIVATION IN MACROPHAGES J774A.1.

N. A. Torres-Aviles[1], L. C. Acosta-Saavedra[1], A. L. Luna[1], E. K. Silbergeld[2] and E. S. Calderon-Aranda[1]. [1]*Department . Toxicologia, Cinvestav, Mexico, Mexico and* [2]*BSPH, Johns Hopkins University, Baltimore, MD.*

DDE(dichloro-diphenyl-dichloroethylene) is the most persistent metabolite of DDT since it has been found in high concentration in the serum of people of region where DDT was used. In macrophages J774A.1 activated with IFN-γ or LPS, p,p'-DDE reduced nitric oxide (NO•-) production and iNOS gene expression, as well as bactericide ability against Mycobacterium microti, suggesting that both pathways share molecules targeted by p,p'-DDE. The iNOS gene transcription is regulated by NFκB and STAT1α,activated by LPS and IFN-γ pathway, respectively, however, it has shown that in dendritic cells, among others, JAK2, a kinase involved in canonical activation of STAT1α, participates in the activation pathway of NF-κB. The objective of this study was to assess whether JAK2 is a molecular target of p,p'-DDE in macrophages activated with LPS, to explain, at least partially, the mechanism of decreasing of NO•- production. Methods In J774A.1 macrophages activated with LPS was evaluated the effect of one hour pre-exposure to 2.5 μg/ml p,p'-DDE on the activation of JAK2, STAT-1α and NF-κB by Western blot, and NO•-, determined by the Griess method. Results shown that the p,p'-DDE inhibits NO•- production in LPS-activated cells and that JAK2 activation is involved in LPS-induced production of NO•-; in non exposed cells LPS induces phosphorylation of JAK2/STAT-1α, while p,p'-DDE in activated cells, over-induced JAK2/STAT-1α, but reduced NF-kB activation induced by LPS,suggesting that this effect is non-dependent of over-activation of JAK2; p,p'-DDE in non activated cells induces JAK2/STAT-1α pathway. Taken together our results show that the inhibition of NO•- production is at least partially dependent of the inhibition of the iNOS gene activation via of NF-κB, but this effect is not related with the effect of p,p'-DDE on JAK2, but more studies about the mechanisms of inhibition of p,p'-DDE in the functional activation of macrophages are needed. This work was supported by Conacyt-46297.

PS 404 A ROLE FOR STRESS-RESPONSIVE SIGNALING IN THE REGULATION OF ERK OSCILLATIONS.

T. J. Weber[1], H. Shankaran[2], K. M. Waters[2], W. B. Chrisler[1] and R. L. Sontag[1]. [1]*Cell Biology & Biochemistry, PNNL, Richland, WA and* [2]*Computational Biology, PNNL, Richland, WA.*

Deregulation of feedback control processes by stress-responsive signaling can play an important role in pathophysiology. We have recently demonstrated that the extracellular signal regulated kinase (ERK) displays persistent nuclear-cytosolic oscillations in response to tyrosine kinase receptor ligands (EGF, bFGF) in mouse and human cell systems. ERK oscillations are inhibited by ≥ 0.32 hydrogen peroxide and 10 cGy X-irradiation, doses that do not produce observable decrease in cell viability using a neutral red assay. Antibiotics used to select for transduced cells expressing selectable markers inhibit ERK oscillations in both reversible (normal immortalized human keratinocytes) and irreversible (primary human keratinocytes) fashion. We confirmed that X-irradiation induces an increase in phospho-p38 levels by Western blot and p38 is frequently activated in response to a broad range of cell stressors. Growth factor-dependent ERK oscillations can be restored in a subset of non-oscillating cells by an inhibitor of p38 kinase (SB203580). Collectively, these observations demonstrate that 1) ERK oscillations are inhibited by hydrogen peroxide and X-irradiation at low dose exposures, 2) ERK oscillations can be restored in non-oscillating cells by a p38 kinase inhibitor and 3) standard practices for maintaining and engineering cells can disrupt ERK oscillations potentially contributing to inter-laboratory biological variability. The disruption of ERK oscillations represents a putative new mode-of action by which toxicological agents can perturb biological systems.

PS 405 HARMINE MODULATES DIOXIN-INDUCED CYP1A1 ENZYME THROUGH TRANSCRIPTIONAL AND POST-TRANSLATIONAL MECHANISMS.

M. El Gendy and A. El-Kadi. *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.*

Dioxins are widespread environmental contaminants that induce the carcinogen-activating enzyme cytochrome P450 1A1 (CYP1A1) through an aryl hydrocarbon receptor (AhR)-dependent mechanism. Harmine is a common β-carboline present in several medicinal plants such as *Peganum harmala*. Harmine possesses antitumor

and cytotoxic effects; however, its effect on dioxin-induced level of CYP1A1 has not been studied before. Therefore, the aim of this work is to study the effect of harmine and its main metabolite, harmol, on dioxin-induced AhR-mediated signal transduction both in mouse and human hepatoma cell lines. For this purpose; HepG2 and Hepa 1c1c7 cells were incubated with increasing concentrations of harmine and harmol (0.5-12.5 μM) in the presence of dioxin (1nM). Our results showed that harmine and harmol significantly inhibited the dioxin-induced CYP1A1 at mRNA, protein and activity levels, in a concentration-dependent manner, in human hepatoma HepG2 cells. However, the effect of harmine was more pronounced. Moreover, harmine and harmol inhibited the induction of CYP1A1 activity induced by two other AhR ligands, namely, 3-methylcholanthrene and β-naphthoflavone. The ability of harmine to affect the induced level of CYP1A1 was strongly correlated with its ability to reduce the AhR-dependent luciferase activity and the electrophoretic mobility shift assay (EMSA). At posttranslational level, both harmine and harmol decreased CYP1A1 protein stability, suggesting that posttranslational mechanism is involved. Furthermore, harmine and harmol inhibited the induction of Cyp1a1 caused by dioxin in mouse hepatoma Hepa 1c1c7 cells. We concluded that harmine and harmol can modulate the dioxin-induced CYP1A1 level at transcriptional and posttranslational levels. Furthermore, these data may represent novel mechanisms by which harmine and its metabolite, harmol, interfere with the dioxin-mediated toxic effects including carcinogenicity. **Acknowledgement:** This work was supported by Natural Sciences and Engineering Research Council of Canada.

PS 406 E7 VIRAL ONCOPROTEIN NEGATIVELY INFLUENCES THE TRANSCRIPTIONAL ACTIVITY OF THE L1MD RETROTRANSPOSON PROMOTER UNDER CONDITIONS OF CELLULAR STRESS.

D. E. Montoya-Durango[1, 2] and K. S. Ramos[1, 2]. [1]*Biochemistry and Molecular Biology, University of Louisville, Louisville, KY and* [2]*Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY.*

LINE-1 (L1) retrotransposons are mobile elements that modify the eukaryotic genome by a copy and paste mechanism that allows them to reinsert elsewhere in the genome to cause disease. L1 activation is observed under conditions of chemical and oxidative stress. We previously identified mouse L1Md-A5 as a redox-regulated genetic element and found both antioxidant and E2F/Rb-binding sites within the 5' promoter region that confer transcriptional activation and repression, respectively. The viral oncoprotein E7 inactivates Rb protein and associates with basal transcription factors including the AP1 family members c-Jun, JunB, JunD, and c-Fos. Since Rb recruits histone deacetylase (HDAC) activity to repress L1 transcription, we investigated (i) the effects of E7 and Rb complex on the mouse L1 5'UTR and (ii) the changes in protein complex formation in primary cells under redox stress conditions. Transient transcription assays in HeLa cells were employed to monitor the effect of forced E7, Rb, and HDAC2 expression on L1MdA-A5 promoter activity following challenge with the carcinogen benzo(a)pyrene (BaP). E7 ablated the response of the reporter gene to carcinogen treatment, but did not interfere with basal transcriptional activity. Rb overexpression transactivated the promoter, but this effect was quenched when E7 alone or in combination with HDAC2 were added. Using gel filtration chromatography we found that in primary vascular smooth muscle cells subjected to BaP treatment, Nrf2 and JunD proteins shift to a macromolecular complex of 1 MDa, while JunD complexes between 700-100 kDa disappear. We conclude that E7 associates with transcription factors required for proper assembly of the transcription macromolecular complexes that assemble on the ARE and E2F/Rb. This is the first report showing that E7 might be involved in the regulation of L1 retroelement activity via both the ARE and E2F/Rb-binding sites.

PS 407 NEOPLASTIC LUNG CELL PROLIFERATION, STIMULATED BY ALVEOLAR MACROPHAGE-DERIVED IGF-1, CAN BE ABROGATED BY THE COMBINED INHIBITION OF MEK AND PI3K.

J. M. Fritz, L. D. Dwyer-Nield and A. M. Malkinson. *Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Rationale: In human non-small cell lung cancer, chronic inflammation resulting from toxin exposure or underlying pathology greatly promotes the growth of initiated cells. In mouse models, tumor growth is enhanced by chemically-induced inflammation. This chronically increases alveolar macrophage infiltration, paralleling that which occurs naturally later in lung tumor progression. These observations suggest that macrophage recruitment stimulates neoplastic proliferation. Herein, we show that macrophage-derived IGF-1 is one factor that significantly promotes tumor growth. Methods: Macrophage conditioned media (MØCM) was generated from freshly isolated mouse bronchoalveolar lavage (BAL) cells. Macrophages were also co-cultured with primary murine tumor cell isolates or stable neoplastic lung epithelial cell lines in *vitro*. Results: MØCM and macrophage co-culture stimulate tumor cell proliferation to similar degrees. IGF-1 is highly abundant in BAL fluid, compared to other cytokines. IGF-1 levels are 3.5x higher in tumor-bearing lungs vs. naïve, and tumor-educated BAL macrophages produce 2x more. Recombinant IGF-1 stimulates neoplastic growth *in vitro* and is additive to MØCM, while other factors such as IL-1β and EGF had no growth effect. Decreasing MØCM IGF-1 levels by siRNA or immuno-depletion reduces the growth stimulation in subsequently treated cells. MØCM and IGF-1 increase Erk and Akt activation in neoplastic cells, resulting in increased cyclin D1 expression and increased proliferation. Combined pharmacologic inhibition of both MEK and PI3K blocks the effects of MØCM and IGF-1, unexpectedly increasing Akt activity, but decreasing cyclin D1 expression consistent with suppressed neoplastic proliferation. Conclusions: Inhibition of MEK and PI3K ablates macrophage-derived IGF-1-induced neoplastic lung cell proliferation but induces hyper-activation of Akt. (Supported by CA132552; Fritz, J.M. is an AFPE Fellow)

PS 408 MASS SPECTROMETRY TECHNIQUES TO STUDY PROTEIN-LIGAND INTERACTIONS AND MOLECULAR TOXICOLOGY PATHWAYS.

K. E. Yamada[1, 2], C. M. Ryan[3], J. P. Whitelegge[3] and C. D. Eckhert[1, 2]. [1]*Molecular Toxicology IDP, University of California Los Angeles, Los Angeles, CA,* [2]*Environmental Health Sciences, University of California Los Angeles, Los Angeles, CA and* [3]*The Pasarow Mass Spectrometry Laboratory, The NPI-Semel Institute, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA.*

Native protein mass spectrometry is an under utilized technique to study protein-protein and protein-ligand interactions. Notable advantages of this method are the ability to study direct binding and the ability to detect molecules without any labeling. Traditional binding experiments usually depend on radio, immuno, or fluorescent labels and mass spectrometry circumvents this requirement by direct mass measurement. Furthermore, identification of specific binding site is possible. Here we look at the effects of boric acid on the interaction of FKBP12 with its endogenous ligand, cADPR. This technique has implications in understanding toxicity mechanisms and pathways on the molecular level. It is sensitive enough to detect picogram quantities of protein and resolve mass differences of less than 1 dalton. Furthermore, advances in spraying conditions allow us to study the effects of ligands on protein conformational changes. Mass spectrometry has the potential to be a very powerful tool for molecular toxicologists.

PS 409 DICLOFENAC INHIBITS TNFα-INDUCED NF-κB NUCLEAR SHUTTLING CAUSING SYNERGISTIC HEPATOCYTE APOPTOSIS *VIA* CASPASE-8.

L. Fredriksson, B. Herpers, Z. Di and B. van de Water. *Toxicology, LACDR, Leiden University, Leiden, Netherlands.*

Drug-induced liver injuries (DILIs) are the major cause of drug failures and are often idiosyncratic in nature. We hypothesize that idiosyncratic DILI occurs due to crosstalk between drug reactive metabolite and cytokine stress signaling. To study this hypothesis, human hepatoma HepG2 cells were exposed to diclofenac, which causes idiosyncratic DILI in humans, in the presence of the pro-inflammatory cytokine TNFα. Diclofenac itself induced a mild concentration-dependent apoptosis of HepG2 cells. While TNFα itself was not cytotoxic, it strongly enhanced the diclofenac-induced apoptosis. Using a siRNA screening approach and a live cell imaging of apoptosis technique, diclofenac/TNFα-induced apoptosis was identified as death-receptor dependent involving the intrinsic, mitochondrial death pathway. In addition, diclofenac itself caused sustained activation of the stress kinase JNK, and knock-down of this gene also resulted in an inhibition of the induced apoptosis. Under normal conditions these two pro-apoptotic signaling pathways that are activated down-stream of the TNF-receptor are controlled by the activation of the transcription factor NF-κB and the resulting gene transcription. By using immunofluorescence staining of wild type HepG2 cells and live cell imaging of HepG2 cells expressing GFP-p65 we show that diclofenac causes a delay in the NF-κB oscillatory nuclear-to-cytosol translocation pattern in association with reduced NF-κB transcriptional activity. The anti-apoptotic role of p65 was evident since both inhibition of IKK as well as stable lentiviral shRNA-based knock down of p65 sensitized hepatocytes towards diclofenac/TNFα-induced cytotoxicity. Together our data suggest a model whereby diclofenac-mediated stress signalling suppresses

TNFα-induced survival signalling routes and sensitizes cells to apoptosis and consequently the onset of DILI. We anticipate that our work will enable us to identify mechanism-based biomarkers that can predict idiosyncratic-like DILI in a pre-clinical drug development setting.

PS 410 NRF2B: A NOVEL NRF2 PARALOG IN ZEBRAFISH.

A. R. Timme-Laragy[1], S. I. Karchner[1], D. G. Franks[1], M. J. Jenny[2] and M. E. Hahn[1]. [1]*Biology, Woods Hole Oceanographic Institution, Woods Hole, MA and* [2]*University of Alabama, Tuscaloosa, AL.*

NF-E2-related factor 2 (Nrf2) regulates antioxidant defenses by activating gene expression via antioxidant response elements (AREs). An Nrf2 ortholog was identified previously in zebrafish (Danio rerio). We have identified and cloned a novel zebrafish Nrf2 paralog, Nrf2b. The predicted protein sequence shares several domains with the original Nrf2 (now Nrf2a), but lacks the neh4 and neh5 transactivation domains. Zebrafish-human comparisons demonstrate conserved synteny between nrf2 and hox genes. nrf2a is on chromosome 9 near the hoxda cluster and nrf2b is on chromosome 6 near the degenerate hoxdb cluster, indicating that nrf2a and nrf2b are co-orthologs of the human NRF2 gene. Nrf2a and Nrf2b display different patterns of expression during embryonic development. While Nrf2a expression increased throughout development (0-120 hours post fertilization; hpf), Nrf2b had the highest expression pre-hatch (48-60 hpf); embryos that hatched early had low expression at these same times. In adults, Nrf2b was most highly expressed in the ovary, female gut, and gill; lowest expression was found in male kidney and eye. In transient transfection assays in COS-7 cells, Nrf2b was less active than Nrf2a, but was capable of activating an ARE-regulated reporter gene in response to tert-butylhydroquinone (tBHQ). No deformities were noted after morpholino knockdown of either Nrf2a or Nrf2b. Nrf2a morphant embryos were more sensitive to tert-butylhydroperoxide (tBOOH) but not tBHQ, whereas knock-down of Nrf2b did not affect sensitivity of embryos to either chemical. Nrf2b expression was increased following exposure to TCDD and PCB-126 in an Ahr2-dependent manner, suggesting crosstalk between Nrf2b and Ahr2 signaling pathways. Further examination of zebrafish Nrf2 co-orthologs may yield new understanding about the evolution, function, and regulation of NRF2.
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PS 411 CRITICAL CYSTEINE RESIDUES OF KEAP1 IN SUPPRESSION OF NRF2 BASAL ACTIVITY AND ARSENIC-SENSING BY REGULATING THE UBIQUITINATION-PROTEASOMAL DEGRADATION OF NRF2 PROTEIN.

X. He[1] and Q. Ma[1, 2]. [1]*Receptor Biology Lab. /TMBB/HELD, NIOSH, Morgantown, WV and* [2]*Biochemistry, West Virginia University, School of Medicine, Morgantown, WV.*

Nrf2 mediates the induction of antioxidant response element (ARE)-dependent drug metabolizing enzyme genes and antioxidative genes by a wide range of structurally divergent inducers including carcinogenic metals such as arsenic. Activation of Nrf2 involves modulation of Keap1/Cul3-mediated ubiquitination-proteasomal degradation of Nrf2. Here we analyzed arsenic-Keap1 cysteine thiol interactions for Nrf2 activation. Fluorescent arsenic FlAsH and phenylarsine oxide (PAO) were used to probe binding of arsenic to Keap1. FlAsH emitted strong fluorescence upon binding to purified Keap1, and arsenic, tert-butyhydroquinone (tBHQ), or the thiol reactive 2, 3–dimercaptopropanol inhibited FlAsH binding. Purified or endogenous Keap1 was effectively pulled down by PAO affinity beads in vitro and from hepa1c1c7 cells, and arsenic, tBHQ, free PAO, or cadmium blocked Keap1 pull down. Furthermore arsenic and free PAO significantly reduced the free thiol contents of purified or endogenous Keap1 in cells. Therefore, arsenic, FlAsH, PAO, tBHQ, and cadmium bind to Keap1 cysteine thiols competitively. All domains of Keap1 bound PAO, but the linker region exhibited highest binding activity. The function of arsenic-Keap1 interaction was evaluated in a reconstituted system that mimics endogenous Nrf2 regulation. Mutation of C273 or C288A in linker region resulted in higher level expression of Nrf2 protein in the absence of inducers. Mutation of C151A abolished Nrf2 activation by arsenic. Overexpression of C273A, C288A, or C151A altered the basal and arsenic-induced expression of Nqo1 consistent with regulated protein levels of Nrf2. The study demonstrates important roles of C273 and C288 in the suppression of Nrf2 by Keap1 under basal conditions and critical function of C151 in arsenic sensing and responsiveness. Our findings support the notion that arsenic binds to different sets of Keap1 cysteine residues to regulate divergent functions in Nrf2 signaling.

PS 412 THE KEAP1-NRF2 SYSTEM REGULATES METALLOTHIONEIN EXPRESSION AND PROTECTS VASCULAR ENDOTHELIAL CELLS FROM CADMIUM CYTOTOXICITY.

Y. Shinkai[1], Y. Kumagai[1], T. Kimura[2], C. Yamamoto[3], M. Yamamoto[4], H. Jinno[5], T. Tanaka-Kagawa[5] and T. Kaji[6]. [1]*Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan,* [2]*Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan,* [3]*Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan,* [4]*Graduate School of Medicine, Tohoku University, Sendai, Japan,* [5]*Division of Environmental Chemistry, National Institute of Health Sciences, Tokyo, Japan and* [6]*Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Japan.* Sponsor: A. Naganuma.

Cadmium, a ubiquitous heavy metal, is an important industrial and environmental pollutant that can target the vascular endothelium. However, the molecular and cellular mechanisms of protection against cadmium toxicity are poorly understood. In the present study, we investigated role of the Keap1-Nrf2 system in cellular response to cadmium and protection against this metal in bovine aortic endothelial cells. Exposure of the cells to cadmium caused Nrf2 activation, resulting in the expression of antioxidant proteins such as heme oxygenase-1, NAD(P)H: quinone oxidoreductase-1, and glutamate cysteine ligase modulatory subunit. We found that siRNA-mediated knockdown of Nrf2 attenuated expressions of not only these antioxidant proteins but also metallothionein-I/II, thereby enhancing cadmium cytotoxicity, whereas siRNA-mediated knockdown of Keap1, the negative regulator for Nrf2, potentiated induction of metallothionein genes, leading to protection of the cells from cadmium cytotoxicity. Moreover, chromatin immunoprecipitation assay showed that Nrf2 was recruited to the antioxidant response element region of the metallothionein gene promoter in the presence of cadmium. Taken together, these results suggest that the Keap1-Nrf2 system serves as a defense mechanism against cadmium toxicity through positive regulation of metallothionein genes in the vascular endothelial cells.

PS 413 MECHANISTIC INVOLVEMENT OF THE NRF2/KEAP1 ANTI-OXIDANT RESPONSE IN THE REGULATION OF HUMAN ABCC3.

M. D. Merrell[1], M. J. Canet[1], F. Zhao[1], T. Wu[1], J. M. Maher[2] and N. J. Cherrington[1]. [1]*Pharmacology and Toxicology, University of Arizona, Tucson, AZ and* [2]*Department of Medical Biochemistry, Tohoku University, Sendai, Japan.*

Members of the ATP-binding cassette C (ABCC) family, including ABCC3, play an important role in toxicology and disease by actively effluxing a wide variety of endogenous and exogenous substrates. Induction of human ABCC3 has been reported during hepatic stress (disease, toxicant exposure) and in the progression of some forms of cancer. Multiple investigations have implicated the transcription factor Nrf2 in this induction, and the functional antioxidant response element (ARE) has been characterized in the mouse ABCC3 gene. However, while recent studies in A549 human lung cells (which over-express Nrf2) have demonstrated a clear role for Nrf2 in the induction of human ABCC3, no functional human response element has been defined. Results of a ChIP-sequencing experiment revealed a specific interaction between Nrf2 and the eighth intron (-26.3kb to -27.5kb) of the human ABCC3 gene, and not the upstream promoter. Subsequent in silico analysis of this intron revealed several putative AREs and ARE-like elements. Additionally, while previously identified putative response elements in the upstream promoter are not evolutionarily conserved, sequence alignment revealed sequence conservation of an intronic ARE across ten eutherian mammal species, including five non-primate species. Luciferase reporter gene assays using an Nrf2 overexpressing cell line (A549) and constructs containing 1.2kb of the eighth intron revealed increased activity over vector-controls. Finally, rtPCR-based chromatin immunoprecipitation using antibodies against Nrf2 confirmed the specific DNA-Nrf2 interaction identified in the ChIP-sequencing experiment. Our findings identifying an Nrf2 response element within an intron of the human ABCC3 gene may provide a mechanistic understanding of the induction of human ABCC3 during the antioxidant response.

PS 414 ATTENUATION OF THE NRF2 SIGNALING PATHWAY IN HEPATOCYTES LACKING SIRTUIN 1 (SIRT1).

W. Wei[1], J. Xu[1], S. Kulkarni[1], X. Li[2] and A. Slitt[1]. [1]*BPS, University of Rhode Island, Kingston, RI and* [2]*National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

Primary hepatocytes are effective tools for in vitro evaluation of biotranformation enzyme and transporter induction. Tertiary butylhydroquinone (tBHQ) is a phenolic antioxidant that stabilizes the protein NF-E2-related factor 2 (Nrf2, Nfe2l2) and enhances antioxidant response element-mediated gene transcription. Sirt1 is a

histone deacetylase that is necessary for peroxisome proliferator activated-receptor alpha mediated gene induction. The purpose of the current study was to determine whether tBHQ induces drug transporter expression in hepatocytes via a Sirt1-dependent mechanism. Primary hepatocytes were isolated from male wild-type or hepatocyte-specific Sirt1 knockout mice (Sirt1-KO) by a two-step perfusion. After plating on collagen-coated plates, cells were treated with tBHQ (100μM) for 24 hours, and then total RNA was isolated. The mRNA expression levels were quantified by quantitative real-time PCR. tBHQ increased Peroxisome proliferator-activated receptor alpha (Ppar-α),Peroxisome proliferator-activated receptor-gamma coactivator-1α(Pgc-1α) as well as Nad(p)h:quinone oxidoreductase(Nqo1) and multidrug resistance-associated protein (Mrp)2,3 mRNA expression in wild-type hepatocytes, but this induction was attenuated in Sirt1-KO hepatocytes. In summary, tBHQ is a known activator of the Nrf2 signaling pathway in primary hepatocytes. This induction was attenuated in the Sirt1-KO hepatocytes, which may be due to decreased Pgc1-α and Ppar-α expression or Sirt1-dependent modulation of Nrf2 activity. (NIH 3R01ES016042)

PS 415 RESPONSE OF NRF2-MODULATED GENES TO CIGARETTE SMOKE.

B. Kosmider, E. Messier, H. Chu and R. J. Mason. *National Jewish Health, Denver, CO.*

Oxidative stress caused by cigarette smoke (CS) directly causes lung injury and cell death in chronic obstructive pulmonary disease. Moreover, many studies show that second-hand smoke can have harmful effects on nonsmokers and even cause them to develop lung cancer and heart disease. The epithelium is the barrier between inhaled air, which contains toxic compounds in cigarette smoke and the underlying tissue. To study CS-induced cell injury we analyzed human primary alveolar type II (ATII) cells and alveolar macrophages isolated from deidentified healthy, nonsmoker, smoker and ex-smoker lung donors. We compared expression of Nrf2, and its repressor Keap1 and Nrf2-dependent genes such as HO-1 and NQO1 in these cells by western blotting, RT-PCR and immunocytofluorescence. We found higher Nrf2 expression in smokers and ex-smokers in comparison with non-smokers. Moreover, we also analyzed Nrf2 localization in ATII cells and in lung tissue. We observed Nrf2 translocation to the nucleus in smokers in comparison with nonsmokers. This suggests Nrf2 activation by reactive oxygen species (ROS) induced by cigarette smoke. These results indicate oxidative stress and DNA damage. Identification of the pathways whereby cigarette smoke generates ROS and induces apoptosis may further our understanding of the pathogenesis of cigarette smoke-induced lung disease. This work is supported by a Young Clinical Scientist Faculty Award to Beata Kosmider from the Flight Attendant Medical Research Institute.

PS 416 INDIRUBIN-3'-(2, 3 DIHYDROXYPROPYL)-OXIMETHER (E804) IS A POTENT AHR-AGONIST AND MODULATOR OF INFLAMMATION PROFILES IN LPS-TREATED RAW264.7 MACROPHAGES.

A. S. Babcock and C. D. Rice. *Biological Sciences, Clemson University, Clemson, SC.*

Multiple indirubins have been synthesized and shown to inhibit cyclin-dependent kinases (CDKs) and glycogen-synthase kinase (GSK-3β) with varying degrees of potency. Several indirubins are also aryl hybrocarbon receptor (AhR) agonists, and as with CDK and GSK-3β inhibitory activities of indirubin derivatives, AhR activities cover a wide range of potency. One indirubin in particular, Indirubin-3'-(2,3 dihydroxypropyl)-oximether (E804) demonstrates novel activity against STAT3 signaling. This is significant because STAT3 signaling is the primary means by which IL-10 mediates its anti-inflammatory actions. Furthermore, IFN-γ induced MHC-II expression and antigen presentation are suppressed by IL-10 through STAT3 signaling. To date, there are no published studies describing the effects of E804 on AhR signaling, nor has this compound been investigated for its potential as an anti-inflammatory agent in macrophage-targeted initiatives to modulate inflammation. We found that E804 induces the expression of CYP1A, AhR, and COX-2 in the murine macrophage cell line RAW264.7 in a manner similar to that induced by PCB-126. Induction of COX-2 by E804 suggests a pro-inflammatory property, therefore the expression of iNOS, IL-6, TNF-alpha, and p65 in cells co-treated with LPS were examined, but found to be suppressed. Using a focus qRT-PCR array, we compared E804 and PCB-126 for their effects on expression of a suite of genes associated with inflammation and toxicity. In most cases those genes up-regulated by LPS treatment are suppressed by E804 and PCB-126. Collectively, these data indicate that E804 is a potent AhR ligand and modulator of proinflammatory profiles in the murine macrophage line RAW264.7 treated with LPS. As with other potent AhR ligands, E804 may exert its effects by altering developmental pathways: in macrophages this may contribute to differences between M1- and M2- like phenotypes.

R 417 REFORMING THE TOXIC SUBSTANCES CONTROL ACT (TSCA): CHALLENGES, OPPORTUNITIES, AND TIMING.

T. Lewandowski[1] and S. Barone[2]. [1]*Gradient, Seattle, WA and* [2]*U.S. EPA, Washington, DC.*

The Toxic Substances Control Act (TSCA) of 1976 significantly changed the regulatory landscape for chemicals in the United States. It gave the federal government greater powers to track the introduction of new chemicals into the consumer marketplace, provided the U.S. EPA with limited powers to require testing of new chemicals, and allowed for case-by-case restriction of chemicals shown to be particularly hazardous. Yet the basic provisions of TSCA are now 35 years old, and unlike most major environmental laws passed in the 1970s, the TSCA legal framework remains essentially unchanged. Achieving the goals of TSCA, namely to ensure that adequate data are available to allow assessment of the effect of chemical substances and mixtures on health and the environment has also proven difficult. The regulatory apparatus has been unable to cope with the large number of chemicals requiring evaluation, has focused on new rather than existing chemicals, has largely ignored the potential interaction of chemicals occurring in mixtures, and has generally failed to keep up with advances in technology. While once a model regulation for other nations, the TSCA framework has now been superseded by chemical safety regulations adopted by other jurisdictions (e.g., REACH). Given the dramatic changes in chemical technology, toxicology, and risk assessment that are expected to occur in the near future, reform of TSCA is seen as a high priority by many stakeholders. Issues that are likely to be addressed by reform of TSCA include the distinct toxicology and exposure scenarios posed by nanotechnology, the possibility of basing hazard identification for the large number of chemicals requiring assessment on mechanistic and in vitro data rather than standard animal tests, and consideration of special population groups (not only children but also those with genetic susceptibilities or chronic health conditions). Reform of TSCA has the potential to greatly affect the way chemical risks are assessed in the U.S. and may therefore have a significant impact on the daily lives of society members.

IS 418 THE INTERNATIONAL COOPERATION ON ALTERNATIVE TEST METHODS (ICATM): TRANSLATING SCIENCE TO PROVIDE IMPROVED PUBLIC HEALTH SAFETY ASSESSMENT TOOLS.

W. Stokes[1] and M. Wind[2]. [1]*NICEATM, NIEHS, Research Triangle Park, NC and* [2]*Consumer Product Safety Commission, Bethesda, MD.*

In 2009, the United States, Canada, the European Union, and Japan signed a Memorandum of Cooperation for International Cooperation on Alternative Test Methods (ICATM). This agreement provides for enhanced international collaborations for the validation, evaluation, and development of internationally harmonized recommendations for new and alternative safety testing methods and strategies. The initial participating validation organizations are the NICEATM and ICCVAM, ECVAM, JaCVAM, and Health Canada's Environmental Health Science and Research Bureau. A Korean Center for the Validation of Alternative Methods (KoCVAM) has recently also been established and is in the process of becoming an ICATM participant. The ICATM organizations work collaboratively to promote the validation and regulatory acceptance of new, revised, and alternative test methods that are based on sound science and that will provide continued or improved protection of people, animals, and the environment while reducing, refining, and replacing the use of animals where scientifically feasible. The participating validation organizations seek to expand and strengthen cooperation, collaboration, and communications on the scientific validation and evaluation of new alternative testing methods proposed for regulatory health and safety assessments. Consistent collaborations are critical to the design and conduct of validation studies, the evaluation and independent scientific peer review of proposed test methods and the development of harmonized recommendations for national and international regulatory consideration. The enhanced international cooperation in these three areas is expected to result in more efficient test method validation and review, and more rapid national and international acceptance of scientifically valid test methods. We will provide an overview of the ICATM process along with an introduction to each participating validation organization and highlights of recent and planned ICATM contributions.

EC 419 SOCIAL MEDIA AND INFORMATICS ESSENTIALS FOR TOXICOLOGISTS.

L. Burgoon[2], S. Bhatia[1], P. Wexler[3] and M. Price[4]. [1]*Human Health Sciences, RIFM, Woodcliff Lake, NJ,* [2]*Biostatistics and Bioinformatics Research Core (BBRC), U.S. EPA, Durham, NC,* [3]*Toxicology and Environmental Health Information Program, National Library of Medicine, Bethesda, MD and* [4]*Membership and Social Media Marketing, Society of Toxicology, Reston, VA.*

How many times a day do we use Google or a search engine? Its use has become so intuitive that we have coined this term as a verb and simply cannot imagine functioning without it. Information that was once confined to libraries and various periodicals is now free and easily accessible since the advent of Internet technology. Furthermore, it has broken cultural and language barriers and enabled one to communicate and collaborate with people all over the world. The combination of social media, open source programs, and bioinformatics has transformed the role of the computer in the modern scientist's life. Video journals, blogging, open access journals, social network websites, and podcasts have become the new channels of communication - enabling first hand transfer of free knowledge and an ease by which to carry out many collaborative efforts. A familiarity with simple Internet searching, word processing, and expansive spreadsheets is simply not an adequate preparation. Furthermore, the software tools used to deal with data arising from in silico models, toxicogenomics, or high-throughput screens require an understanding of basic concepts in computer science, database design, bioinformatics, and statistics. To bridge the gap between these two worlds our panel of experts will provide toxicologists with the basic knowledge of the informatics and various open source tools available. In closing, we'll discuss innovative strategies including the use of social media as a communication, collaboration, networking, and a career advancement tool.

PS 420 DEVELOPING A SCREENING SYSTEM FOR HEPATOTOXICITY BY COMBINING THE HEPARG CELL MODEL WITH HIGH CONTENT IMAGING.

M. Mennecozzi, M. Tsaneva, L. Saavedra and M. Whelan. *Sistems Toxicology Unit, European Commission-Joint Research Centre, Ispra, Varese, Italy.* Sponsor: D. Dix.

Although it is evident that liver toxicity is still a major issue in the pharmaceutical sector, there is still no established in vitro screening system to reliably identify potentially hepatotoxic drug candidates early in the development phase. Moreover, in the safety assessment of industrial chemicals and cosmetic ingredients, tighter restrictions on the use of animals has generated considerable demand for alternative methods for assessing systemic toxicity, with hepatotoxicity being a central element of any resulting testing strategy. It is clear that hepatotoxicity encompasses a range of complex processes and toxicological pathways, and thus it is a major challenge to capture the key biological events in a screening system. We have addressed the problem by combining a highly relevant cellular model, HepaRG, with high content imaging. The HepaRG cell line is derived from a human hepatocellular carcinoma and when seeded at low density, differentiates into bipotent hepatic progenitors and divides before acquiring morphological and functional characteristics of human hepatocytes. Differentiated HepaRG cells express the major liver functions, including P450s, phase II enzymes, transporters and nuclear receptors at levels comparable to those found in primary hepatocytes. The high content imaging approach we adopted is based on automatic analysis of image-sets acquired with an epifluorescent microscope, for the quantification of biomarkers expressed by treated HepaRG cells. A quantitative high throughput screening format was employed using a 96-well plate format, which facilitates the testing of 80+ chemicals in one experimental run. We exposed HepaRG cells to serial concentrations of chemicals/drugs and analyzed multiple cellular phenotypic changes including cell loss, nuclear size, nuclear morphology, DNA content, mitochondrial membrane potential, and intracellular accumulation of lipids. Dose-response data were acquired and analyzed to characterize both the technical performance of the system and its ability to predict hepatotoxicity.

PS 421 USING PRECISION-CUT LIVER SLICES TO PROFILE BIOCHEMICAL CHANGES OF DRUG INDUCED HEPATOTOXICITY.

R. Hernandez, J. W. Lawrence and M. A. Lafleur. *Amgen, Thousand Oaks, CA.* Sponsor: S. Sawant.

Precision cut liver slices (PCLS) in dynamic organ culture provide a model that has the potential to improve in vitro hepatotoxicity testing since it contains all hepatic cell types and native matrix. Moreover, we have demonstrated that the use of certain culture medium constituents, such as glucocorticoids, are not required. Glucocorticoids are potent anti-inflammatory agents and prevent a vital portion of liver reactions to hepatotoxicants. We have developed the PCLS model into a useful tool to rapidly profile various pathways involved in toxicity. Several endpoints (LDH release, alkaline phosphatase, glycogen, triglycerides, cholesterol, TBARS, glutathione, ATP content, and caspase induction) were measured for a series of agents with known hepatic toxicity liabilities and several non-hepatotoxic agents. The biliary epithelial cell toxicants, ANIT and 17-AAG, had selective effects by reducing slice alkaline phosphatase levels without affecting LDH release. Troglitazone was demonstrated to have oxidative stress-inducing activities on GSH, TBARS, and NO induction. Agents that affected energy metabolism involving mitochondria reduced ATP and glycogen and often reduced glutathione content at concentrations that did not release LDH. Using this system, agents with known oxidative stress components, energy metabolism changes, and cholangiolytic specific toxicities can be distinguished. This system allows researchers to profile several pathways in parallel that are important to hepatic toxicity induction and prioritize more resource demanding activities in the laboratory.

PS 422 CYTOTOXICITY OF 3-(3, 5-DICHLOROPHENYL)-2, 4-THIAZOLIDINEDIONE (DCPT) AND ANALOGUES IN WILD TYPE AND CYP3A4-TRANSFECTED HEPG2 CELLS.

D. M. Frederick, E. Y. Jacinto, T. H. Rushmore, R. Tchao and P. J. Harvison. *Department of Pharmaceutical Sciences, University of the Sciences, Philadelphia, PA.*

The thiazolidinedione (TZD) ring is found in the glitazones, drugs used in the treatment of type II diabetes. Liver injury has been reported in the clinic following use of these drugs; however, they do not produce hepatotoxicity in common laboratory animal species. In contrast, 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione (DCPT) causes hepatic damage in rats. Previous in vivo work has shown that DCPT hepatotoxicity is dependent on an intact TZD ring and cytochrome P450 (CYP)-mediated metabolism. To further study the role of biotransformation in TZD ring-induced toxicity, DCPT and several analogues were tested in vitro using wild type HepG2 cells and HepG2 cells that were stably transfected with CYP3A4. Troglitazone (TGZ), rosiglitazone and 5-(3,5-dichlorophenylmethyl)-2,4-thiazolidinedione (5-DCPMT), a compound with a similar TZD substitution pattern to the glitazones, were also tested. CYP3A4 transfectants were characterized by an enzyme marker assay (6β-hydroxylation of testosterone; Km = 81.1 μM, Vmax = 92.7 pmol/min/mg protein). Both cell lines were treated with 0-250 μM of each compound in Hanks' balanced salt solution (HBSS; 0.1% DMSO). After 24 hours, cell viability was measured. Some of the compounds did not exert cytotoxicity in either cell line. However, DCPT was significantly ($p < 0.05$) more toxic in CYP3A4 transfectants than wild type cells at or above 200 μM. In contrast, 5-DCPMT and TGZ exerted greater cytotoxicity in the wild type cells, but only at intermediate concentrations. Median lethal concentrations (LC50s) for DCPT (transfectant LC50 = 152.5 μM; wildtype LC50 = 206.8 μM), 5-DCPMT (transfectant LC50 = 19.4 μM; wild type LC50 = 13.8 μM) and TGZ (transfectant LC50 = 24.6 μM; wild type LC50 = 21.0 μM) were consistent with these findings. Based on these results, we conclude that all three parent compounds are toxic and that CYP3A4 may contribute to DPCT-induced cytotoxicity in the transfectant cells. Supported by PHS grant ES012499.

PS 423 DEVELOPMENT OF AN ANIMAL MODEL OF AMODIAQUINE-INDUCED LIVER INJURY.

P. Cai, T. Nakgawa and J. Uetrecth. *Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.*

Background: Amodiaquine (AQ) is an antimalarial drug that is no longer used for prophylaxis because it is associated with life-threatening agranulocytosis and/or hepatotoxicity. AQ forms a reactive iminoquinone metabolite and these idiosyncratic reactions appear to be immune-mediated, but how this reactive metabolite might induce an immune response is unknown. AQ is reported to cause acute liver toxicity in rats at high dose; the purpose of this study was to develop a model of AQ-induced idiosyncratic hepatotoxicity at low dose that is delayed similar to the reaction in humans. Methods and Results: Male Wistar or Brown Norway (BN) rats were treated with AQ at 62.5 mg/kg/day, 6 days/week for 5 weeks via gavage. Serum ALT was increased in both Wistar (65.8 U/L ±3.2 in treated vs 26.7 U/L ± 4.2 in controls) and BN (65.5U/L ± 6.7 in treated vs 36.8 U/L ±3.0 in controls) rats after 4 weeks of treatment, which then returned to almost normal despite continued treatment. Pathological changes of activated macrophages in the lung, activated Kupffer cells and lymphocyte infiltration in the liver were found. The number of ED1 or ED2 stained Kupffer cells was significantly increased in both rat strains, but the increase was greater in BN rats. Apoptotic cells were found in the liver of BN rats. Co-treatment with poly I:C (TLR 3), one dose of 10 mg/kg or imiquimod (TLR 7) one dose 30 mg/kg, appeared to lead to an earlier onset of ALT increase while co-treatment with LPS (TLR 4) once a week 5 mg/kg appeared to delay the onset, but in all cases the ALT returned toward normal despite continued treat-

ment. Paradoxically, buthionine sulfoximine, which inhibits glutathione synthesis, prevented the AQ-induced ALT increase. Cyclosporine also decreased AQ-induced ALT elevation. Conclusion: An AQ-induced liver injury model was developed in rats that is characterized by a delayed onset of mild liver injury, activated Kupffer cells/macrophages, apoptotic cells and lymphocyte infiltration in the liver. The effect of cyclosporin is consistent with an immune mechanism. This work was supported by grants from CIHR.

PS 424 DEVELOPMENT OF AN ANIMAL MODEL OF ISONIAZID-INDUCED LIVER INJURY.

I. G. Metushi[1] and J. P. Uetrecht[2, 1]. [1]*Pharmacology, University of Toronto, Toronto, ON, Canada and* [2]*Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.*

Background: Idiosyncratic drug reactions are a major medical problem. A classic example is isoniazid (INH)-induced liver toxicity. Previous studies suggested that this toxicity is due to bioactivation of acetylhydrazine based on the observation that no covalent binding of the parent drug was observed and acute toxicity in an animal model was prevented by inhibition of the hydrolysis of N-acetylisoniazid. However, it seemed unlikely that INH would not also be oxidized to a reactive metabolite. We set out to determine if INH is oxidized to a reactive metabolite and to develop an animal model with characteristics more like the liver injury that occurs in humans. Methods and Results: An anti-INH antibody was generated by reacting Protein Blue with an active ester of isonicotinic acid followed by immunization of rabbits with this antigen. This antibody has a high affinity for isonicotinic acid-modified protein and it detected many modified proteins from the livers of rats treated with INH on a western blot. Attempts were made to develop a mouse model by treating C57BL and Cbl-b (-/-) mice with INH (200 mg/kg/day in food) and co-treated with either INH-modified hepatic protein, 6-formylindolo[3,2-b]carbazole or anti-CD25 antibody to try to prevent immune tolerance. Although these treatments did not lead to an increase in ALT, we found that INH interferes with the ALT assay by reacting with pyridoxal, the cofactor in the assay, so we changed to a sorbitol dehydrogenase (SDH) assay to test for liver injury. Treatment of Brown Norway rats with INH (200 or 400 mg/kg/day by oral gavage) for 1 week led to an increase in serum SDH levels. Conclusions: These studies demonstrate that bioactivation of INH itself leads to covalent binding and further work is being carried out to determine the mechanism of injury in this animal model and to see if it is consistent with INH-induced liver injury in humans. This research was supported by grants from CIHR.

PS 425 APPROACH FOR DETECTING FLUTAMIDE-INDUCED HEPATOTOXICITY USING GLUTATHIONE DEPLETED RATS.

H. Horie, I. Miyawaki, I. Matsumoto, T. Kai, M. Aoki, T. Yamada, J. Kimura, H. Funabashi and T. Seki. *Dainippon Sumitomo Pharmacology Co., Ltd., Osaka, Osaka, Japan.*

AIM: Flutamide, a nonsteroidal antiandrogen drug primarily used for treatment of prostate cancer, is associated with idiosyncratic drug-induced liver injury (DILI) in human patients. In pre-clinical phase, however, it is known that obvious hepatotoxicity is not found in flutamide-treated rodents. Glutathione (GSH) plays an important role in protection against oxygen stress and reactive hepatotoxic metabolite. Generally the glutathione s-transferase activity of rodents is higher than that of human, which may be contributing the species difference in incidence of DILI. In this study, we aimed to detect flutamide-induced hepatotoxicity using hepatic GSH depleted rats. METHODS: For depletion of hepatic GSH, we used 2 GSH-depleting agents, D,L-buthionine sulfoximine (BSO) and diethyl maleate (DEM). Sprague-Dawley male rats were intraperitoneally injected with BSO-DEM mixture (500 mg/kg each in corn oil) or vehicle 1 h before flutamide treatment. Flutamide (300 mg/kg in 0.5% methylcellulose) or vehicle was orally treated by gavage. Rats were sacrifice 6 and 24 h after treatment of flutamide to collect serum and liver samples. Measurement of serum hepatic enzyme activities, hepatic GSH level and gene expression, and histopathological evaluation of the liver were carried out. RESULTS: Slight centrilobular vacuolation in the hepatocytes was noted by flutamide treatment, and the hepatic alteration was markedly accelerated by depletion of hepatic GSH (ex. centrilobular vacuolation and necrosis in the hepatocytes, increases in AST, ALT and LDH). Expression of GSH synthesis-related genes were increased by GSH depletion, and the changes were accelerated by flutamide treatment. Expression of oxidative stress-related genes was also increased by flutamide treatment. CONCLUSIONS: We could successfully detect flutamide-induced hepatotoxicity using GSH-depleted rats. Thus, it is considered that the GSH-depleted rat could be useful to detect DILI potential of candidate drugs in the pre-clinical phase.

PS 426 A 3-DIMENSIONAL LIVER MODEL FOR THE SAFETY-EVALUATION OF DRUG CANDIDATES.

R. M. Kostadinova, F. Boess, L. Suter-Dick, T. Weiser, T. Singer and A. Roth. *F. Hoffmann-La Roche Ltd., Basel, Switzerland.*

Drug-induced liver injury is a common adverse effect observed in animal studies and the main cause for acute liver failure and post-marketing drug withdrawals. Because of species-specific differences in hepatocellular biology, animal experiments can predict liver toxicity in man only to a certain extent. We therefore see an urgent need to apply in vitro assays using human cell models to support and qualify data generated in animal experiments to assess the potential human response. Variety of human 2D monolayer cell-culture systems are used in drug-toxicity screens. However, all of these systems do not accurately recapitulate liver structure, physiology and function, thus limiting the value of prediction of liver toxicity for humans. We have functionally characterized rat and human in vitro 3-dimensional liver models (3D) from RegeneMed and demonstrated that cells grown on a 3D scaffold maintain liver function over a period of several months, as measured by the secretion of specific markers such as albumin, fibrinogen, transferrin and urea. We found that the drug-inducible metabolic activity of key members of Cytochrome P450s was preserved for several months in 3D liver cells. Using cell-type specific protein markers, we demonstrated by immunocytochemistry that, like in adult liver, in vitro 3D liver co-cultures contained hepatocytes, hepatic stellate cells, kupffer cells and endothelial cells. The presence of different cell types allows measuring the release of cytokines upon treatment with an inflammatory stimulus. To assess the utility of the 3D liver system for toxicity screening, we monitored the toxicity of hepatotoxins over time and found that the human and rat 3D models have the potential to better detect drug-induced liver toxicity as compared to 2D hepatocytes. In conclusion, liver cells with preserved heterotypic cell-cell interactions and 3D liver architecture, appear to resemble in vivo liver physiology and metabolism and we propose that this new model could be used in the future for more accurate evaluation of drug-induced liver toxicity.

PS 427 A BENCHMARK DRUG LIST FOR THE STUDY OF DRUG-INDUCED LIVER INJURY.

V. Vijay, M. Chen, Q. Shi and W. Tong. *Systems Biology, NCTR/U.S. FDA, Jefferson, AR.*

The identification of relevant biomarkers to predict Drug-Induced Liver Injury (DILI) is very challenging. An accurate, reliable, and reproducible approach to classify known drugs corresponding to their DILI potentials is essential for comparative analysis to identify key DILI mechanisms and risk factors. Such studies could result in the discovery of biomarkers useful in drug development and regulatory review. Currently, drug classification for DILI potential has primarily relied on case reports and consensus among pathologists. These processes are both time-consuming and difficult for independent groups to accurately reproduce, and thus have limited potential to be adopted as common practices for annotating drugs to support perspective studies. The objective of this study is to develop a benchmark dataset for DILI study in order to validate existing or novel DILI biomarkers and to predict DILI potential for new compounds. Using drug labels that are drafted by the manufacturers and approved by the FDA, we developed a systematic and objective classification scheme for the DILI potential of drugs. This effort is a central component of the Liver Toxicity Knowledge Base (LTKB) project initiated at the FDA's National Center for Toxicological Research. We developed an LTKB benchmark dataset (LTKB-BD) that contains 155 DILI associated drugs and 65 non-DILI associated drugs, which will grow over the years as the LTKB project progresses. All of these drugs are commercially available, and each has over 10 years of market use. The label information for all these drugs is stable, evidence of their DILI potential is well-established, and our annotation procedure can be reproduced by others to augment the list. In addition, the LTKB-BD has good representation of known drugs in terms of chemical structure, therapeutic categories, and daily dosage. In summary, the LTKB-BD has the potential to serve as a benchmark dataset for the scientific community to evaluate the performance characteristics of existing DILI biomarkers and to develop novel DILI biomarkers using emerging molecular technologies.

PS 428 MONOCYTE TO MACROPHAGE DIFFERENTIATION-ASSOCIATED 2 (MMD2) IS A NEW CANDIDATE GENE FOR HEPATOPROTECTIVE EFFECT OF TAMOXIFEN AGAINST DRUG-INDUCED LIVER INJURY.

T. Yokoi[1], Y. Yoshikawa[1], Y. Toyoda[1], S. Higuchi[1], T. Tsukui[2], K. Tsuneyama[3], T. Fukami[1] and M. Nakajima[1]. *[1]Graduate School of Medical Science, Kanazawa University, Kanazawa, Ishikawa, Japan, [2]Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Saitama, Japan and [3]Department of Diagnostic Pathology, Toyama University, Toyama, Toyama, Japan.*

Drug-induced liver injury (DILI) is one of the major problems in drug development and clinical drug therapy. However, the underlying mechanism in DILI is not well understood. The administration of ERα agonists, such as estradiol and ethinylestradiol (EE2), is known to cause both aggravate and protect against liver injury, suggesting that ERα is involved in liver injury. In this study, DILI caused by acetaminophen (APAP), bromobenzene (BB), diclofenac (DIC), and thioacetamide (TA) were suppressed by tamoxifen (TAM) pretreatment in female mice. DNA microarray analyses suggested that monocyte to macrophage differentiation-associated 2 (Mmd2), whose function was unknown, could be a candidate gene for liver protection. To investigate the Mmd2 function in DILI, Mmd2 mRNA was knocked down in mice by injection of siRNA for Mmd2 (siMmd2). A significant decrease of Mmd2 mRNA to about 30% was obtained. In Mmd2 knockdown and TAM-pretreated mice, TA-induced liver injury was exacerbated. In addition, administration of TAM to ERα knockout mice did not change the Mmd2 mRNA level, suggesting that Mmd2 was regulated via ERα. In TAM-pretreated mice, amphiregulin (Areg: known as a gene for liver regeneration) mRNA was increased, suggesting that the one of the mechanisms of the liver protective function of Mmd2 was liver regeneration via the increase of Areg. In contrast to mouse, MMD2 mRNA was hardly expressed (0.1% of mouse) in human liver. This is the first report of the involvement of ERα in DILI, and the hepatoprotective mechanism of Mmd2 induction via ERα was also elucidated.

PS 429 INVESTIGATING LIVER MITOCHONDRIAL TOXICITY USING PRIMARY RAT HEPATOCYTES AND A LIVER CELL LINE.

C. Summers, K. Abbott, J. I. Goodman and A. Foster. *Safety Assessment, Astrazeneca, Alderley Park, Cheshire, United Kingdom.* Sponsor: D. Brown.

Drug-induced liver injury (DILI) makes up a large proportion of adverse drug reactions. Mitochondrial impairment is a key mechanism of DILI and members of a broad range of drug classes demonstrate mitochondrial liabilities. Aims of the present study were to identify an *in vitro* alternative to isolated hepatocytes for routine mitochondrial toxicity testing, and to develop the mitochondrial component of a high content imaging (HCI) assay for cytotoxicity. We showed that the SV40 T-antigen immortalized human liver epithelial (THLE) cell line cultured in 7mM glucose for 24hrs, exhibited increased resistance to classical mitochondrial inhibitors (FCCP, oligomycin and antimycin), compared with rat hepatocyte sandwich cultures (RHSC), as measured using an ATP cytotoxicity assay (Sigma-Aldrich ATP bioluminescent assay kit). In contrast, THLE cells cultured in 7mM galactose for 24hrs, displayed increased sensitivity to the same mitochondrial inhibitors, compared to RHSC. Mitochondrial physiology was studied by fluorescent labeling of the cells with tetramethyl rhodamine ethyl ester (TMRE) and nuclei were stained with Hoechst. Image acquisition was performed on an ImageXpress Micro®, and the MetaXpressTM granularity algorithm was employed to measure changes in mitochondrial staining. HCI revealed differences in mitochondrial staining between the cell types in the absence of compound treatment. With the RHSC, HCI was able to detect mitochondrial toxicity prior to general cytotoxicity measured by ATP depletion. In contrast, in the THLE cells, the dose dependent decrease in mitochondrial staining paralleled the ATP results, except for the FCCP-treated cells in glucose. These data show that THLE cells in galactose, in conjunction with THLE cells in glucose, provide a viable system for *in vitro* identification of mitochondrial toxicity. TMRE staining of the THLE cell line could be included as a mitochondrial component in a hepatotoxicity assay. HCI provides additional information to traditional cytotoxicity assays, for investigative studies in RHSC.

PS 430 *IN VITRO* METHOD DEVELOPMENT TO ASSESS THE ROLE OF KUPFFER CELLS IN DRUG-INDUCED HEPATOTOXICTY.

N. Shangari[1], M. Dong[2], A. Reising[1], O. Weber[2], E. Cruz[1], S. Sampath[1], P. Patel[1], M. Gurnani[1], C. Buono-Dalton[4], V. Richard[4], T. Wang[3], M. Uteng[2], J. Scheumann[2], S. Chibout[2], F. Pognan[2] and A. Wolf[2]. *[1]iTOX, Preclinical Safety, Novartis Institutes of Biomedical Research, East Hanover, NJ, [2]iTOX, Preclinical Safety, Novartis Institutes of Biomedical Research, Basel, Switzerland, [3]Preclinical Safety, Novartis Institutes of Biomedical Research, Emeryville, CA and [4]iTOX, Preclinical Safety, Novartis Institutes of Biomedical Research, Cambridge, MA.*

Kupffer cell (KCs)-mediated toxicity is a major concern during development of new drug candidates. Here we present an in vitro strategy to assess KC involvement in drug-induced toxicity. Precision-cut liver slices (PCLS) and hepatocyte (HCs)-KC co-cultures were used to assess KC-mediated toxicity of ABC123 (Novartis development compound). HCs and HC-KC co-cultures were treated with ABC123. While ABC123 was not cytotoxic in HCs when treated for 48 hours with upto 300 μM, the same concentration caused significant ALT/AST increases in the HC-KC co-cultures. In KC 300 μM ABC123 increased the release of IL-1α and IL-1β. Furthermore, HC treated with the cytokines, IL-1α, IL-1β or TNFα, together with 300 μM ABC123 showed increased ALT/AST values. ABC123 when incubated with rat PCLS for 24 hours caused significant ALT/AST increases, ATP depletion and hepatic necrosis (seen by histopathology) at 250 to 500 μM. When KCs were depleted or impaired in function, either by gadolinium chloride (GdCl3) or a non-toxic dose (100 μM) of ABC123 (CSF-1R inhibitor), 24 hours before being exposed to toxic (500 μM) levels of ABC123, cytotoxicity was inhibited and inflammatory cytokines IL-1α, IL-1β, TNFα and nitrate levels were reduced in PCLS by the low ABC123 concentrations. These results suggest that depleting or inactivating KCs protect against ALT/AST elevations might play a role in the mechanism of the in vitro cytotoxicity. These in vitro models may serve as tools to understand the role of KC-mediated hepatotoxicity.

PS 431 AUTOMATED IMAGING, QUANTIFICATION, AND CLASSIFICATION OF NF-KAPPA B AND NRF2 RESPONSES TO IDENTIFY ADVERSE DRUG REACTIONS IN HEPG2 CELLS.

B. Herpers, L. Fredriksson, Z. Di and B. van de Water. *Division of Toxicology - LACDR, Leiden University, Leiden, Netherlands.*

Adverse drug reactions often coincide in the liver, due to xenobiotics metabolism. Inadequate responses to drug metabolite stress and periods of inflammation are proposed to synergize in case of idiosyncratic drug-induced liver injury (DILI), causing loss of liver cell function and promoting cell death. To quantitatively follow the different critical events that define this model, we set up live cell imaging and image quantification methods in human hepatoma (HepG2) cells, to monitor Nrf2 stabilization, NF-κB nuclear translocation oscillations and apoptosis under drug and cytokine (co-)exposure conditions. Using this system, we classified the cellular responses to a panel of compounds linked to idiosyncratic DILI (e.g. diclofenac, ketoconazole, carbamazepine) under co-challenge with cytokines (e.g. TNFα and interleukin-1β). Analysis of the image sequences revealed compound-specific onset and rate of GFP-Nrf2 stabilization. Pre-exposure with the various hepatotoxicants interfered with cytokine-induced NF-κB nuclear translocation responses by delaying the NF-κB oscillation pattern. These drug-cytokine synergies can ultimately accelerate the HepG2 cell death (either through apoptosis and/or necrosis). For mechanistic insight, we are currently successfully applying functional genomics (siRNA) screens to identify the signaling pathways and the crucial genetic responses in relation to gene expression profiling. We identified TNFAIP3 and CYLD as opposing factors for the control of NF-κB-induced genetic responses that decide for cell survival or cell death under drug-cytokine synergy conditions.

PS 432 MECHANISTIC STUDIES ON THE CYTOTOXICITY OF LEFLUNOMIDE AND ITS MAJOR METABOLITES IN RAT HEPATIC CELLS.

Q. Shi, X. Yang and W. F. Salminen. *Division of Systems Biology, U.S. FDA, NCTR, Jefferson, AR.*

Leflunomide, a dihydroorotate dehydrogenase inhibitor with immuno-modulatory, anti-proliferative and anti-inflammatory activities, was approved by the U.S. FDA to treat rheumatoid arthritis in 1998. In July 2010, the U.S. FDA required that a boxed warning statement be added to the drug label indicating the potential of severe liver injury. This study aimed to investigate the mechanisms underling leflunomide-induced liver injury. Primary rat hepatocytes were treated with the parent

drug leflunomide and its major (A77 1726) and minor (4-trifluoromethylaniline) metabolites and cytotoxicity was determined at different time points. Leakage of lactate dehydrogenase indicated that leflunomide was significantly more cytotoxic than A77 1726 at concentrations ranging from 250 μM to 1000 μM, while 4-trifluoromethylaniline was essentially non-toxic. Similar rank order was obtained with other toxicity endpoints such as the decrease of mitochondrial membrane potential and reduction of cellular ATP, both of which showed a much earlier onset than cell killing. Further studies using chemical inhibitors of cytochrome P450s (CYP) showed that the cytotoxicity of both leflunomide and A77 1726 were remarkably enhanced by CYP inhibition. These findings were reproduced in clone-9 cells, an immortalized cell line of rat liver origin. These data imply that an individual with mitochondrial abnormalities or decreased drug metabolizing capability might be more susceptible to leflunomide-induced liver injury.

PS 433 THE HEMOSTATIC SYSTEM AND NEUTROPHILS IN AMIODARONE/LIPOPOLYSACCHARIDE-INDUCED LIVER INJURY IN RATS.

J. Lu[1], R. A. Roth[2] and P. E. Ganey[2]. *[1]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI and [2]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Amiodarone (AMD), a class III antiarrhythmic drug, causes idiosyncratic hepatotoxicity in human patients. We demonstrated previously that an inflammatory stress caused by lipopolysaccharide (LPS) renders otherwise nontoxic doses of AMD hepatotoxic in rats. In this study, we investigated the roles of the hemostatic system and neutrophils in this model. Male, SD rats were treated with a nonhepatotoxic dose of AMD or its vehicle ip and 16 hrs later with lipopolysaccharide (LPS) or saline iv. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were elevated only in AMD/LPS-cotreated rats. Plasma concentrations of thrombin-antithrombin complex (TAT) and plasminogen activator inhibitor-1 (PAI-1) were elevated after LPS administration, and AMD cotreatment significantly prolonged the duration of the increases, suggesting that AMD enhanced hemostasis caused by LPS. Analysis of immunostained liver sections showed that AMD/LPS cotreatment increased fibrin deposition in the liver. Administration of anticoagulant heparin attenuated the ALT activity increase caused by AMD/LPS. LPS administration increased hepatic neutrophil (PMN) accumulation, and cotreatment with AMD slightly decreased the number of PMNs in livers of LPS-treated rats. However, PMN activation was detected only in AMD/LPS-cotreated animals. Depletion of circulating PMNs with rabbit anti-rat PMN serum attenuated the AMD/LPS-induced increase in ALT activity. In summary, hemostatic system impairment and PMN activation were observed after AMD/LPS cotreatment, and anticoagulation or PMN depletion attenuated AMD/LPS-induced liver injury. (Supported by NIH R01DK061315.)

PS 434 STUDY OF LIVER LYMPHOCYTES IN AMODIAQUINE-INDUCED HEPATOTOXICITY IN BROWN NORWAY RATS.

F. Liu[1], P. Cai[1], J. Li[2] and J. Uetrecht[1]. *[1]Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada and [2]Immunotoxicology, Bristol-Myers Squibb Company, New Brunswick, NJ.*

Background: Amodiaquine (AMQ) is an antimalarial drug associated with severe adverse drug reactions, including hepatotoxicity and agranulocytosis. The mechanism of this drug-induced toxicity is still unknown. We found that treatment of Brown Norway rats with AMQ (62.5 mg/kg/day), it induced mild delayed onset hepatotoxicity that may be represent an animal model for the milder forms of AMQ-induced liver injury in humans. This injury was accompanied by hepatic macrophage activation, a mild lymphocytic infiltration, and hepatocyte apoptosis. There was also proliferation of NK cells and Th17 cells in the spleen and lymph nodes. This study will help us get a better understanding on the mechanism of localized liver injury induced by AMQ. Methods and Results: Liver lymphocytes were isolated from liver after 5 weeks of AMQ treatment at the dose of 62.5 mg/kg/day. Phenotyping by flow cytometry was employed to identify Th17 (Il-17+/CD4+), NK (CD3-CD161+), NKT cells (CD3+CD161+) and CD8+ T cells, respectively. The results demonstrated that AMQ treatment induced more IL-17 producing cells (1.33±0.34%) in the liver compared to control rats (0.46±0.26%); and also Th17 cells increase from 0.06±0.02% to 0.62±0.40% after AMQ treatment; similarly, NK cells increased from 33±4% to 54±17% after AMQ treatment. No significant difference was observed for CD3+ T cells, CD4+ T cells, CD8+ T cells and NKT cells. Conclusion: The present study demonstrates that AMQ-induced liver injury in rats is associated with proliferation of Th17 cells and NK cells in the liver. This suggests that this injury is immune-mediated and that these may be the effector cells for this injury. This research was supported by grants from the Canadian Institutes of Health Research.

PS 435 SIGNIFICANT RELATIONSHIP EXISTS BETWEEN ORAL DRUG EXPOSURE LEVEL AND ITS POTENTIAL RISK FOR HUMAN HEPATOTOXICITY.

M. Chen, V. Vijay and W. Tong. *Division of Systems Biology, U.S. FDA/NCTR, Jefferson, AR.*

Many drugs that have been withdrawn from the market or have received black box warning were prescribed at "high" doses. It has been found that there is a relationship between daily dose of oral medications and idiosyncratic hepatic adverse events. This suggests that drug's potential risk of hepatotoxicity was related to its exposure level in humans. It is well-known that the critical drug exposure level is the drug concentration in the plasma and the target organ/cell, but not the administered dose. In this study, we assessed maximum drug concentration in the plasma (i.e. Cmax) and its relationship with drug's potential risk of hepatotoxicity using six largest published datasets, each contained more than 100 oral medications. In these datasets, each drug was labeled according to its potential association with hepatotoxicity, such as little risk, low risk or high-risk. Drug's daily dose was obtained from the WHO's defined daily dose (DDD) database, and Cmax were manually curated from the PubMed and other sources. Using independence test and logistic/ordinal regression, it was found that daily dose was significantly associated with drug's potential risk of hepatotoxicity across 5 datasets except one dataset, while Cmax was significantly associated in all 6 datasets. Furthermore, we used Receiver Operating Characteristic (ROC) analysis to assess the statistical performance of daily dose and Cmax. The results indicated that the Area Under Curves (AUCs) of Cmax in different datasets were relatively higher than those of daily dose, and their improvement ranged from 0% to 10%. In summary, our study confirmed the significant relationship of oral drug exposure level with the potential risk for human hepatotoxicity and found that Cmax was a better indicator than daily dose as a DILI signal with better statistical performance.

PS 436 EVALUATION OF PHOSPHOLIPIDOSIS-INDUCING POTENTIAL OF DRUGS FROM ORAL SINGLE-DOSE RAT LIVER GENE EXPRESSION PROFILING.

T. Kai[2], H. T. Yudate[1], Y. Minowa[3], M. Aoki[4], T. Yamada[5], T. Kimura[4], A. Ono[6], H. Yamada[3], Y. Ohno[7] and T. Urushidani[3, 8]. *[1]Strategic Planning & Management, Dainippon Sumitomo Pharmacology Co., Ltd., Osaka, Japan, [2]Genomic Science Laboratories, Dainippon Sumitomo Pharmacology Co., Ltd., Osaka, Japan, [3]Toxicogenomics-Informatics Project, National Institute of Biomedical Innovation, Osaka, Japan, [4]Research Planning & Management, Dainippon Sumitomo Pharmacology Co., Ltd., Osaka, Japan, [5]Safety Research Laboratories, Dainippon Sumitomo Pharmacology Co., Ltd., Osaka, Japan, [6]Division of Risk Assessment, National Institute of Health Sciences, Tokyo, Japan, [7]National Institute of Health Sciences, Tokyo, Japan and [8]Department of Pathophysiology, Doshisha Women's College of Liberal Arts, Kyoto, Japan.*

Purpose Phospholipidosis is an excessive accumulation of phosphospholipids in tissues. Drug development can be delayed if phospholipidosis is observed in preclinical and clinical trials. We therefore aim to discover novel biomakers that can be used at the lead optimization stage for prioritizing compounds.
Methods We used liver gene expression data at the 24 hours time point after single-dose administration of each of 6 phospholipidosis-positive compounds and 21 negative compounds to male SD rats. A genetic algorithm was applied to optimize a set of markers, which was subsequently validated on all compounds from the Japanese Toxicogenomics Project (TGP) and most recently on independent test compounds.
Results & Discussion We optimized a set of 25 gene markers which were able to completely classify the training set of 27 compounds. To validate our model, the phospholipidosis-inducing potential of 161 TGP compounds were assessed. We found the set of markers can properly evaluate the potential of compounds not used for training. Moreover, the set of markers also classified the independent test compounds correctly. Our marker set represents an early time-point gene-expression pattern that may eventually lead to phospholipidosis. Because phospholipidosis can only be detected in histopathology examination after an extended period of repeated dose administration, this predictive marker set is valuable for phospholipidosis screening early in the drug development process.

PS 437 EVALUATION OF 3 BILE ACIDS AS POTENTIAL BIOMARKERS OF DRUG-INDUCED LIVER INJURY.

L. Luo, C. A. Fritz and J. L. Colangelo. *Drug Safety R&D, Pfizer Inc., Groton, CT.*

Adverse drug reactions, in particular drug-induced liver injury (DILI), represent a major challenge for safe drug development. Increases in total circulating bile acid concentrations have been utilized as indicators of hepatotoxicity. These measurements do not portray the dynamic changes of the individual bile acids. Accurate

measurement of individual bile acids has biological significance with regard to determining their role in hepatotoxicity and liver disease. Previous studies, in which 21 individual bile acids (IBA) were evaluated, demonstrated that 3 IBA were potential biomarkers of DILI in the rat model. To investigate these further, an LC/MS/MS method to quantitate the 3 IBA of taurocholic acid, glycocholic acid and cholic acid was optimized and validated. Three deuterated internal standards used for the method and very good accuracy, precision, linearity and reproducibility were achieved. Serum samples from rats dosed with known toxicants, including hepatotoxicants, cardiac toxicants, and skeletal muscle toxicants, were analyzed and quantitated for the three bile acids. Results were evaluated against histopathology findings, clinical chemistry parameters and other proposed biomarkers. The concentration of the bile acids were observed to increase significantly in the animals dosed with all hepatotoxins on fold change and p-value, but not statistically increased in other toxins dosed animals relative to control animals, even alanine amimotransferase (ALT) elevated. Also, cholic acid was observed to response prior to the onset of ALT elevations in rats dosed with hepatotoxins. The data indicated that the bile acids can potentially be an earlier, more specific and more sensitive biomarker of hepatotoxicity in preclinical species. The relationships of the individual bile acids to the specific disease states and their predictivity will be further investigated.

PS 438 UNTARGETED METABOLOMICS IDENTIFIED A PANEL OF MARKERS FOR DRUG-INDUCED LIVER INJURY.

L. Guo[1], N. Masutomi[2], M. Miyake[2], M. Yamazaki[2], H. Sato[2], K. A. Lawton[1], M. V. Milburn[1] and J. A. Ryals[1]. *[1]Metabolon, Durham, NC and [2]Mitsubishi Tanabe Pharmacology Corporation, Chiba, Japan.*

Drug-induced liver injury (DILI) is a major cause for compound attrition during drug development and drug withdraws from market. The current practices using histopathological analyses in preclinical animal models may have limitations in sensitivity and discordance with effects in humans. In order to identify biomarkers for improved DILI detection, we performed an untargeted metabolomic profiling of plasma, urine, and liver samples from rats treated with 13 known liver toxicants at two doses (a low dose and a high dose) and two time points. Nine of the toxicants (acetaminophen, methapyrilene, ticlopidine, bendazac, ethionine, cyclosporine A, naphthylisothiocyanate, tetracycline, and carbon tetrachloride) caused various types and degrees of heptocellular damages and altered clinical chemistry, especially at the high dose and at the later time point. The other 4 toxicants (carbamazepine, flutamide, chlorzoxasone, and nimesulide) did not cause any apparent liver injury to the rats, thus they were grouped as "human specific toxin" as they were known to induce liver injury in human. Statistical analysis and pathway mapping of the nearly 1,900 metabolites detected across the three matrices revealed various diverse metabolic perturbations from each toxicant. A small panel of plasma and urine biomarkers was found to show common responses to most or all the toxicants, including the human specific toxins. The changes of these biomarkers were apparently specific to DILI but not to non-specific metabolic interferences such as cellular energetic state and altered gut flora, thus they could be potentially used as DILI biomarkers during preclinical stage.

PS 439 EVALUATION OF ALT ISOZYMES AS REFINED BIOMARKERS OF DRUG-INDUCED LIVER DAMAGE: CHARACTERIZATION AND DEVELOPMENT OF METHODS FOR ANALYZING ALT1 AND ALT2 ISOZYMES.

M. S. Mondal[1], J. Gabriels[1], K. Zhu[2] and F. Pognan[3]. *[1]Investigative Toxicology, Preclinical Safety, Novartis Institute of Biomedical Research, Inc., Cambridge, MA, [2]Analytical Sciences, Novartis Institute of Biomedical Research, Inc., Cambridge, MA and [3]Investigative Toxicology, Preclinical Safety, Novartis Institute of Biomedical Research, Inc., Basel, Switzerland.*

In clinical and preclinical practices, the total serum alanine aminotransferase (ALT) activity serves as a routine surrogate marker for liver injury. However, serum ALT activity can sometimes be elevated upon drug treatment without detectable liver histopathology findings. Conversely, it is also possible to observe histopathologic liver damage in vivo, with no sign for elevated total ALT activity in serum. In drug development, these uncertainties undermine the diagnostic value of total serum ALT activity measurement, creating risks for false positive or false negative evaluations. To improve upon these uncertainties, we have conducted an investigation over a wide range of organs of the two isozymes - ALT1and ALT2 – that are expressed differentially in various tissues and serum. Using large-scale two dimensional gel electrophoresis (2DGE), Westerns/Mass spectrometry methodologies and functional activity measurements in various tissues, we have established specific methods to detect and identify ALT1 and ALT2 isozymes. Based on our analysis, we propose to correlate the total ALT activity with the levels of ALT1 and ALT2 isozymes in various tissues and serum. Furthermore, in this presentation, we discuss how ALT1 and ALT2 levels and their activities could be used as a possible specific signature for liver damage and how these analyses can help diagnose the mechanisms of ambiguous liver injury.

PS 440 SORBITOL DEHYDROGENASE AND GLUTAMATE DEHYDROGENASE ARE NOT SUPERIOR TO TRADITIONAL BIOMARKERS OF LIVER INJURY: A HEALTHY VOLUNTEER STUDY OF HEPARINS.

A. H. Harrill[1], S. Eaddy[1], J. Roach[2], I. D. Fier[2] and P. B. Watkins[1, 3]. *[1]University of North Carolina-Hamner Institute for Drug Safety Sciences, The Hamner Institutes, Research Triangle Park, NC, [2]Momenta Pharmaceuticals, Cambridge, MA and [3]University of North Carolina Chapel Hill, Chapel Hill, NC.*

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are routinely incorporated into clinical drug development as markers of liver injury. However, some agents, such as heparins, cause ALT and AST elevations, yet rarely, if ever, cause serious liver injury. Sorbitol dehydrogenase (SDH) and glutamate dehydrogenase (GLDH) have been proposed as alternative biomarkers of liver injury and, if specific for serious liver injury, should not be elevated by heparins. To test this hypothesis, we measured SDH and GLDH in serial serum samples obtained in 48 healthy adult subjects who received one of four heparin preparations (s.c.) twice daily for 4.5 days: unfractionated heparin (150 IU/kg, n=12), enoxaparin sodium (100 IU/kg, n=12), dalteparin sodium (120 IU/kg, n=12), or M118 (a novel LMWH - 125 IU/kg, n=12). Blood was collected on study Days -1 (baseline), 1, 2, 3, 4, 5, 6 and 11. Mean serum ALT and AST levels increased following treatment and peaked on Day 6 (mean fold change over subject baseline ± SEM across all treatments for ALT: 7.8 ± 0.6, and AST: 5.9 ± 0.5). At each time point, there was no significant difference (P>0.05) in the serum ALT or AST level observed between each drug tested. Mean serum levels of GLDH and SDH also increased during treatment with each heparin, and peaked on Day 6 (mean fold change ± SEM for GLDH and SDH were 14.4 ± 2.5 and 6.2 ± 0.5, respectively). Fold change in GLDH and SDH were significantly correlated with fold change in ALT levels at all time points; on Day 6, Spearman's rank correlation coefficients were 0.76 and 0.86 for GLDH and SDH respectively. We conclude that GLDH and SDH are not superior to ALT as biomarkers for serious liver injury potential in this model. Efforts are ongoing to use this sera bank to identify liver specific biomarkers that are not elevated by heparins, including miRNA and purine nucleoside phosphorylase.

PS 441 IMPORTANCE OF IL-6 SIGNALING FOR MITOCHONDRIAL STAT3 EXPRESSION AND MITOCHONDRIAL FUNCTION IN MOUSE LIVER.

F. Boess, A. Roth, K. Schad, C. Zihlmann, M. Bellot, A. Olaharski, L. Suter, S. Platz, T. Weiser, T. Singer and L. Mueller. *F. Hoffmann-La Roche AG, Basel, Switzerland.*

Signal Transducer and Activator of Transcription-3 (STAT3) is an important downstream effector in the signaling pathway of several cytokines, including interleukin-6 (IL-6), an important target for the treatment of various inflammatory diseases. In addition to its function in cell growth or apoptosis, a potential novel function of the serine-phosphorylated STAT3 as an essential player for the proper function of the mitochondrial respiratory chain has been proposed by Wegrzyn et al. (2009). As IL-6 is one of the major factors regulating STAT3 activity, the aim of this study was to investigate signaling of mitochondrial STAT3 and mitochondrial function in a situation of absence of IL-6 in order to support safety assessment of therapies targeting IL-6. Method: Liver tissue was harvested from IL-6 knockout (B6.129S2-Il6tm1Kopf) and wild type-mice (C57/B6) at 6, 16 and 30 weeks of age. Protein levels of STAT3, including phosphorylated forms were analyzed in total tissue extracts and subcellular fractions (nucleus, mitochondria, cytosol). In addition, freshly isolated liver mitochondria were used for fluorescence-based live monitoring of mitochondrial oxygen consumption.
Results: Protein expression of STAT3, Ser727-pSTAT3 or Tyr705-pSTAT3 in whole liver extracts was unchanged in WT compared to KO animals at all ages. Analysis of STAT3 and its phosphorylated forms in subcellular fractions of liver tissues did not suggest significant differences in distribution and expression in cytoplasm, mitochondria or nucleus between genotypes and across different ages. Measurements of the respiratory function of liver mitochondria under various conditions revealed no differences between mitochondria form WT and KO animals.
Conclusion: The absence of IL-6 in IL-6 KO animals does not lead to any changes in STAT3 expression or phosphorylation status and does not alter mitochondrial function. Hence, absence of IL-6 signaling does not impair mitochondrial function under normal conditions.

PS 442 GENE EXPRESSION AND MORPHOLOGICAL ANALYSIS OF HEART AND LIVER FROM IL-6 KO MICE.

A. Roth, F. Boess, J. Hoflack, C. Landes, M. Bellot, A. Olaharski, L. Suter, S. Platz, T. Weiser, T. Singer and L. Mueller. *F. Hoffmann-La Roche AG, Basel, Switzerland.*

Mice with a deletion of the Interleukin-6 gene (IL-6 KO) were developed in 1994 by Kopf et al. and have been intensively studied, predominantly under conditions of immunological challenge. IL-6 emerged as an important target for the treatment of various inflammatory diseases. The fact that IL-6 KO mice do not display an altered phenotype under normal conditions, supported Il-6 as a suitable drug target with small inherent risk of undesired adverse effects. A recent publication by Banerjee et al. (2009), however, suggested that IL-6 KO mice undergo severe morphological changes in the heart already early in life. To follow-up on these data we aimed to further examine potential changes in heart and liver induced by deletion of IL-6 with respect to morphology and gene expression. Method: IL-6 KO (B6.129S2-Il6tm1Kopf) and C57/B6 wildtype (WT) mice were sacrificed at 6, 16 and 30 weeks of age, and heart and liver tissue was harvested to allow for histopathological evaluation and microarray gene expression analysis. Results: No macro- or microscopic morphological differences could be found between KO and WT mice. Especially the marked morphological alterations in heart tissue published by Banerjee et al. were not present in this study. No obvious differences in gene expression were observed between IL-6 KO and age matched WT mice. In particular, there was no significant effect of IL-6 deletion at any of the time points analyzed with respect to gluconeogenesis, acute phase response, mitogenesis, apoptosis, or tissue regeneration – processes well-known to be regulated by cytokines such as IL-6. Conclusion: In contrast to recently published work, in the present study absence of IL-6 in IL-6 KO animals did not lead to gross changes in heart or liver under normal conditions, i.e. in the absence of an inflammatory challenge. Blockage of IL-6 signaling is thus considered not to lead to overt adverse changes in these organs, consistent with the view of alternative regulatory mechanisms compensating for the lack of IL-6.

PS 443 TROVAFLOXACIN ENHANCEMENT OF LPS-MEDIATED SIGNALING IS ASSOCIATED WITH REDUCED SOCS-1 EXPRESSION.

R. Singhal, K. L. Poulsen, P. E. Ganey and R. A. Roth. *Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Trovafloxacin (TVX), a fluoroquinolone antibiotic, has been associated with idiosyncratic hepatotoxicity in patients. We previously demonstrated that pretreatment with TVX potentiated lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)α production and led to TNFα-dependent hepatotoxicity in mice. Furthermore, TVX enhanced LPS-stimulated upregulation of TNFα mRNA and protein in RAW 264.7 transformed macrophage cells. Inhibition of p38 phosphorylation or NFkB activation abolished TVX-potentiated LPS-induced TNFα mRNA expression. We hypothesized that TVX pretreatment alters LPS-mediated signaling by suppressing toll-like receptor 4 (TLR4) negative regulators such as SOCS-1, TOLLIP, SIGIRR and SARM. TVX (100 μM) treatment reduced basal and LPS-induced SOCS-1 mRNA expression in RAW cells. It also caused a marginal reduction in TOLLIP mRNA in RAW cells but did not affect transcript levels of SIGIRR or SARM. Treatment of mice with TVX (150 mg/kg) reduced the hepatic levels of SOCS-1 at 3 hr post-treatment without affecting expression in spleen. These results raise the possibility that TVX enhancement of LPS-induced hepatotoxicity and TNFα production is mediated, in part, by reducing SOCS-1 and thereby potentiating TLR4 proinflammatory signaling. (Supported by NIH grant R01DK061315.)

PS 444 ENHANCED ACETAMINOPHEN HEPATOTOXICITY IN VANIN-1 NULL MICE IS INDEPENDENT OF DIFFERENCES IN BASAL HEPATIC GLUTATHIONE LEVELS AND GENE EXPRESSION OF DRUG METABOLIZING ENZYMES AND TRANSPORTERS.

D. W. Ferreira[1], P. Naquet[2], F. Galland[2] and J. E. Manautou[1]. [1]*Pharmaceutical Sciences, University of Connecticut, Storrs, CT and* [2]*Centre d'Immunologie de Marseille-Luminy CNRS-INSERM-Université de la Méditerranée, Marseille, France.*

The Vanin-1 gene encodes a membrane-bound pantetheinase involved in the production cysteamine, a potent antioxidant. Previous transcriptome analysis showed a marked induction of hepatic Vanin-1 gene expression in association with the hepatoprotective effect of the peroxisome proliferator clofibrate. Our recent studies show that Vanin-1 knockout mice are more susceptible to acetaminophen (APAP) hepatotoxicity. Here we investigate whether differential APAP hepatotoxicity in the absence of Vanin-1 is due to differences in APAP bioactivation, disposition or detoxification. Livers of naïve Vanin-1 null and wild type male mice were analyzed for gene expression of APAP metabolizing enzymes and transporters by RT-PCR. No differences in hepatic gene expression of phase I and II enzymes (Cyp1a2, 2e1, 3a11, Ugt1a6) or transporters (Abcc2, Abcc3, Abcc4) were detected between genotypes. Markers of oxidative stress (Hmox1) and inflammation (Tnfα, Cxcl1) were also unchanged. Gene expression of the catalytic subunit of gamma glutamylcysteine synthetase was slightly increased in Vanin-1 null mice. However, no differences in hepatic total glutathione (GSH) concentrations were detected. In mice treated with APAP (400mg/kg, i.p.), induction of Hmox1 was more dramatic in Vanin-1 null mice (~8 fold) compared to wild types (~4 fold) at 24 hours, indicating higher hepatic oxidative stress response in these mice. Collectively, these data suggest that the higher susceptibility of Vanin-1 mice to APAP hepatotoxicity is unlikely due to differences in APAP bioactivation, disposition or basal GSH concentration. Future studies will investigate the role of the Vanin-1 catalytic product cysteamine in APAP hepatotoxicity.

PS 445 DIFFERENTIAL HEPATIC EXPRESSION OF ID GENES BY ACETAMINOPHEN TREATMENT IN MICE.

X. Gu and J. E. Manautou. *University of Connecticut, Storrs, CT.*

Hepatotoxicity by acetaminophen (APAP) is one of the leading causes of acute liver failure in the United States. Hepatocellular damage by APAP is followed by a recovery phase in which normally quiescent hepatocytes replicate to replace necrotic cells. During this regeneration phase, rodents exhibit resistance to toxic APAP reexposure, a phenomenon known as autoprotection. Inhibitor of DNA binding (ID) genes encode a family of helix-loop-helix (HLH) proteins that lack a basic DNA-binding domain and inhibit transcription of basic HLH (bHLH) transcription factors through formation of heterodimers incapable of binding to DNA. ID proteins could play a role in cell growth, senescence and differentiation. To examine potential changes in expression of Id genes during APAP hepatotoxicity and autoprotection, mice were treated with either 400 mg/kg APAP only or APAP (400 mg/kg) followed by a second dose of 600 mg/kg 48 hr later (APAP autoprotection dosing regimen). Id gene expression was examined over a 72 hrs time course. ID gene expression was also examined in vitro in human HC04 liver cells exposed to several proxidant chemicals. The results show that mouse liver Id1 and Id3 gene expression decreased by 2-3 folds at 2 hrs, followed by a 6-10 fold increase between 4 to 12 hrs after a single dose of APAP, returning to normal values by 24 hrs. Similarly, increases in Id2 and Id4 gene expression were detected at several time points after single APAP treatment. With the APAP autoprotection dosing regimen, Id1 gene expression increased 3.8 and 5.6 fold at 4 and 24 hrs, respectively, while induction of Id2 and Id3 gene expression (3-2 fold) was only detected at 4 hrs. No changes in Id4 were detected. Treatment of HCO4 cells with APAP, tBHP or tBHQ also resulted in induction of ID gene expression over time. Collectively, expression of the ID/Id family of genes increases in response to both a single dose of APAP and the autoprotection dosing regimen in mice and in vitro in response to oxidative stress. Supported by NIH DK069557.

PS 446 PROFILING THE HEPATIC PROTEOME TO EXPLORE THE MOLECULAR BASIS OF THE CHRONOTOXICITY OF ACETAMINOPHEN.

P. J. Starkey Lewis[1], C. E. Goldring[1], V. Platt[1], A. M. Obeng[1], L. Randle[1], C. Rowe[1], J. Moggs[2] and K. Park[1]. [1]*Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, Merseyside, United Kingdom and* [2]*Investigative Toxicology, Novartis, Basel, CH-4132, Switzerland.* Sponsor: D. Mendrick.

The mammalian biological clock has been found to exert a powerful influence on the physiology of mammalian systems. This regulation hinges on the complex interplay between the clock genes and their products that oscillate over a twenty-four hour period and promote a diurnal variation in numerous output pathways. Emerging evidence suggests that the efficacy and toxicity of many therapeutic compounds follow a diurnal rhythm and that this may be at least partly attributable to the clock-mediated regulation of drug targets and pathways of drug metabolism. In this study, we have confirmed a significant circadian variation in acetaminophen-induced liver injury in the mouse (CD-1). We have utilized conventional bioanalytical techniques to explore differences in hepatic glutathione status and several CYP450s at the protein and activity levels to begin to address the basis for this variation. Moreover, we have now carried out a more global profile of the circadian hepatic proteome using mass spectrometry, which enabled the determination of the relative levels of approximately four hundred hepatic proteins across a 12h daylight

period. We have identified proteins implicated in bioactivation and detoxification pathways of acetaminophen and other relevant pharmaceutical agents that follow a diurnal variation in expression (e.g. cytochrome b5, cytokeratin-18, peroxiredoxin-6, aromatic-L-amino-acid decarboxylase and regucalcin). This dataset reveals new areas of investigation and testable hypotheses with which to explore the temporal relationship between circadian physiology and DILI.

PS 447 ACUTE LIVER TOXICITY OF ACETAMINOPHEN IS NOT POTENTIATED IN HCV TRANSGENIC MICE.

T. Uehara[1], O. Kosyk[1], E. Jeannot[1], B. Bradford[1], J. Grimes[2], T. O'Connell[2], G. Boorman[3], S. Melnyk[4], S. Weinman[5] and I. Rusyn[1]. *[1]Department of Environmental Sciences and Engineering, UNC, Chapel Hill, NC, [2]The Hamner Institutes, Research Triangle Park, NC, [3]Covance, Princeton, NJ, [4]University of Arkansas for Medical Sciences, Little Rock, AR and [5]University of Kansas Medical center, Kansas City, KS.*

The global burden of hepatitis C virus (HCV) infection is increasing and chronic liver disease in subjects positive for HCV is becoming a significant public health concern in the developed and developing countries alike. While HCV infection may result in steatohepatitis, cirrhosis and hepatocellular carcinoma, it has been shown recently that it also potentiates the hepatotoxicity of acetaminophen (APAP), a drug which is a leading cause of acute liver toxicity. Since the mechanisms of such potentiation are not known, we hypothesized that HCV-Tg mice may be more susceptible to APAP hepatotoxicity and that an animal model may be established to elucidate the mechanisms for co-morbidity. Mice expressing core, E1, E2 and p7 proteins (HCV-Tg) and wild-type C57BL/6J mice were treated with a single dose (300 mg/kg) of APAP and liver toxicity was evaluated at 4 and 24 hrs after dosing. Acute liver injury due to APAP was not exacerbated in HCV-Tg mice and there were few differences between wild type and HCV-Tg in markers of oxidative stress, inflammation, or ER-stress in the liver. Interestingly, the amount of mitochondrial total and reduced glutathione was elevated in HCV-Tg mice, and was decreased upon APAP treatment, an effect not observed in wild-type mice. This study shows that the extent of liver disease in HCV-Tg mice over-expressing core, E1, E2 and p7 alone are not sufficient to produce the sensitivity to APAP that has been observed clinically. However, our findings move the field forward by narrowing down the possibilities of how HCV affects drug toxicity. Our data suggest that either the nonstructural viral proteins are critical for the hyper-sensitivity to drug-induced liver injury in humans, or that it may be due to an immune component that is difficult to reproduce in HCV-Tg mice.

PS 448 INTRAVITAL IMAGING OF ACETAMINOPHEN (APAP) HEPATOTOXICITY.

J. Hu[1], V. K. Ramshesh[1], H. Jaeschke[2] and J. J. Lemasters[1]. *[1]Medical University of South Carolina, Charleston, SC and [2]University of Kansas Medical Center, Kansas City, KS.*

APAP overdose causes liver injury involving mitochondrial dysfunction and onset of the mitochondrial permeability transition (MPT). NIM811 (N-methyl-4-isoleucine cyclosporin) is a nonimmunosuppressive derivative of cyclosporin A that inhibits the MPT *in vitro* and *in vivo*. Monitoring mitochondrial function in living animals has been difficult. Recent developments in intravital confocal/multiphoton microscopy provide a novel approach to visualize mitochondrial dysfunction and cell death *in vivo*. Here we have two aims. The first is to investigate whether NIM811 decreases APAP-induced liver toxicity. Our second aim is to understand the mechanisms of APAP hepatotoxicity and the effect of APAP on mitochondrial polarization and cell death *in vivo*. Male C57BL/6 mice were fasted overnight and then administered APAP (300 mg/kg, i.p.) or vehicle. NIM811 was gavaged (10 mg/kg) 1 h before APAP. For imaging, the abdomen of each mouse was opened, and the exposed liver was positioned on a #1.5 glass coverslip mounted on the inverted stage of an Olympus FV1000 multiphoton microscope. Mitochondrial polarization and cell death were assessed from the respective green and red fluorescence of rhodamine 123 (Rh123) and propidium iodide(PI) using 820-nm multiphoton excitation and a 25X, 1.1 NA water-immersion objective lens. After vehicle, green Rh123 fluorescence was punctate in most hepatocytes, indicating mitochondrial polarization, and nuclear PI staining signifying cell death was absent. At 6 h after APAP, loss of mitochondrial Rh123 fluorescence occurred in pericentral hepatocyes often accompanied by PI labeling. NIM811 decreased both mitochondrial depolarization and cell death. In conclusion, NIM811 attenuates APAP-induced mitochondrial depolarization and necrotic cell death in mouse liver *in vivo*. These results also illustrate the utility of intravital confocal/multiphoton microscopy as a powerful technology to study disruption of liver function in living animals due to APAP.

PS 449 THE ALPHA MUPA MICE, A MODEL OF CALORIC RESTRICTION, ARE MORE SUSCEPTIBLE TO ACETAMINOPHEN HEPATOTOXICITY.

Z. Fu[1], Y. Zhang[1], R. Miskin[2] and C. D. Klaassen[1]. *[1]Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS and [2]Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel.*

Murine urokinase plasminogen activator (αMUPA) transgenic mice spontaneously eat less and live longer (approximately 20%), therefore, they have been considered a caloric restriction (CR) model. Previous studies indicate that CR protects against the hepatotoxic effects of bleomycin and thioacetamide, whereas two other long-lived models, GHR-KO and Snell dwarf mice, are more susceptible to acetaminophen hepatotoxicity. The goal of the present study was to examine the resistance of αMUPA mice to acetaminophen (APAP). Male αMUPA mice and wild-type (WT) mice (14-months of age) were given a single dose of APAP (375 mg/kg, ip). Whereas 92% of WT mice were alive at 48h, the survival of αMUPA mice was only 58% at 24h, and 29% at 48h. In αMUPA mice, APAP caused more severe liver injury, as indicated by higher serum levels of alanine aminotransferase (4.3-fold) and lactate dehydrogenase (5.7-fold), as well as more severe liver necrosis. In unchallenged αMUPA and WT mice, the mRNAs of Cyp2e1 and Cyp3a11, two enzymes important for converting APAP to its toxic intermediate, NAPQI, were similar. However, αMUPA mice exhibited higher mRNAs of some other phase-I drug metabolizing enzymes, such as Cyp1a1 (2-fold), Cyp1a2 (1.6-fold), and Cyp4a14 (2-fold). In addition, αMUPA mice exhibited higher mRNAs of phase-II enzymes that detoxify APAP, Ugt1a1 (1.6-fold) and Ugt1a6 (1.6-fold); that detoxifies NAPQI, Gstp (2.9-fold); and hepatic transporters that transport xenobiotics and bile acids into liver, such as Oatp1a1 (1.6-fold) and Ntcp (1.7-fold). In summary, the present study shows that αMUPA mice exhibit similar expression of key enzymes for APAP bioactivation, and higher expression of enzymes for detoxifying APAP and NAPQI. Therefore, the higher susceptibility of αMUPA mice to APAP hepatotoxicity is likely due to differences in toxicodynamics, rather than toxicokinetics. (Supported by NIH grants ES-09649, ES-09716, ES-07079, DK-081461, and RR-021940)

PS 450 ACTIVATION OF CASPASES DURING ACETAMINOPHEN TOXICITY IS A STRAIN DEPENDENT PHENOMENON.

H. Jaeschke, M. Koerner and C. D. Williams. *University of Kansas Medical Center, Kansas City, KS.*

Acetaminophen (APAP)-induced liver injury resulting from overdose is the most frequent cause of acute hepatic failure in the US. The mechanisms of APAP-mediated cell death have been extensively characterized in both laboratory animals and man and it is generally accepted that liver cells die by oncotic necrosis (Gujral et al., Toxicol Sci. 2002;67:322-8). Recently it was demonstrated in fed but not in fasted CD-1 mice (outbred strain) that there is transient caspase activation after APAP overdose. It was speculated that apoptosis is limited in APAP hepatotoxicity due to the use of fasted animals (Antoine et al., Toxicol Sci. 2009;112:521-31). To evaluate these findings in more detail, APAP-induced liver injury in an outbred strain (Swiss Webster, SW) was compared with the more commonly used inbred mouse strain (C57BL/6). In these studies fed and fasted mice were treated with 530 mg/kg APAP for 3, 5 and 24h with or without pan-caspase inhibitor (10 mg/kg Z-VD-fmk). As a positive control for caspase-dependent apoptosis, mice were treated with 700 mg/kg galactosamine (GalN) and 100 μg/kg endotoxin (ET). Fasted or fed APAP-treated C57BL/6 mice showed no evidence of caspase-3 processing by western blot or caspase-3 activity by Ac-DEVD-AFC fluorogenic assay at any time-point. Interestingly, a minor, temporary increase in caspase-3 processing and enzyme activity (250% above baseline) was observed after APAP treatment in SW mice, but this could be observed in both fed and fasted animals. Z-VD-fmk in vivo partially attenuated this caspase activation but did not alter injury (plasma ALT, area of necrosis). The degree of caspase-3 processing and activation in SW mice after APAP was much less than that observed in the GalN/ET-treated mice (1700% above baseline). Conclusion: Caspase-3 processing and activation after APAP-overdose can be observed only in some outbred mouse strains independent of the fed or fasted state. In contrast to the GalN/ET-induced apoptosis, this transient, minor caspase activation does not seem to impact the overall liver injury and toxicity during APAP overdose.

PS 451 INHIBITION OF THE MITOCHONDRIAL MEMBRANE PERMEABILITY TRANSITION PORE PROTECTS AGAINST ACETAMINOPHEN-INDUCED MITOCHONDRIAL OXIDANT STRESS AND LIVER INJURY *IN VIVO* .

A. Ramachandran[1], M. Lebofsky[1], C. Baines[2], S. A. Weinman[1], J. J. Lemasters[3] and H. Jaeschke[1]. [1]*Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS,* [2]*University of Missouri, Columbia, MO and* [3]*Medical University of South Carolina, Charleston, SC.*

Acetaminophen (APAP) hepatotoxicity is the main cause of acute liver failure in the US and many other countries. Formation of a reactive metabolite, depletion of glutathione and protein binding are hallmarks of APAP-induced cell injury. Mitochondrial dysfunction with formation of reactive oxygen and peroxynitrite has also been implicated in APAP-induced hepatocellular necrosis. However, the impact of these mitochondrial events in influencing APAP-induced cellular signaling and the mitochondrial permeability transition (MPT) pore opening is not well understood in vivo. We used a genetic approach to study the role of cyclophilin D, a critical protein involved in the formation of the regulated MPT. Treatment of wild-type mice, with 200 mg/kg body weight APAP resulted in moderate liver injury in wild type mice at 6 h as indicated by significantly elevated ALT levels (890 +/- 324 U/L), focal centrilobular necrosis and nuclear DNA fragmentation. Ppif(-/-) mice, which lack cyclophilin D, were completely protected against liver injury and DNA fragmentation. Wild type mice also showed elevated levels of hepatic glutathione disulfide, an indicator of reactive oxygen formation, and immunostaining for nitrotyrosine protein adducts, a foot-print for peroxynitrite, in the centrilobular areas after APAP treatment. Both effects were significantly blunted but not eliminated in Ppif(-/-) mice. In contrast, mice partially deficient in the mitochondrial SOD2(+/-) showed a severe aggravation of liver injury. These data indicate that mitochondrial oxidative stress and induction of the mitochondrial permeability transition pore involving cyclophilin D are critical steps in APAP hepatotoxicity in vivo. The mitochondrial SOD2 provides significant protection against APAP-induced MPT and cell necrosis.

PS 452 ROLE OF THE NALP3 INFLAMMASOME IN NEUTROPHIL ACTIVATION AND CELL INJURY DURING ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

C. D. Williams[1], P. J. Shaw[2] and H. Jaeschke[1]. [1]*University of Kansas Medical Center, Kansas City, KS and* [2]*New York University, New York, NY.*

Acetaminophen (APAP) overdose is the leading cause of acute liver failure in the US. Several recent studies imply APAP-induced injury is partially mediated by interleukin-1β (IL-1β) signaling through IL-1-receptor (IL-1R) which can activate and recruit neutrophils, exacerbating injury. After cellular injury, sterile inflammation can lead to inflammasome formation which activates caspase-1 to generate mature IL-1β. The objective this investigation was to evaluate the role of the NALP3 inflammasome and IL-1R signaling on hepatic neutrophil accumulation and liver injury (ALT, necrosis) after APAP overdose. IL-1β mRNA and plasma protein are elevated after APAP overdose. To determine the role of the NALP3 inflammasome in APAP-induced liver injury, mice deficient for each component of the NALP3 inflammasome (Caspase-1, ASC and NALP3) were treated with 300 mg/kg APAP; these mice had similar neutrophil recruitment and liver injury as APAP-treated C57Bl/6 controls. To confirm these findings, IL-1R-deficient mice showed no altered injury or hepatic PMN recruitment after APAP. To test if higher levels of IL-1β can exacerbate APAP-injury, animals were treated with 20 μg IL-1β/kg 3h after APAP. This treatment significantly increased PMN recruitment into the liver by 30% but did not alter liver injury. Evaluation of PMN activation (CD11b expression and reactive oxygen formation by flow cytometry) after APAP or IL-1β treatment showed that only high doses of IL-1β but not APAP alone caused moderate activation of PMNs isolated from the peripheral blood and the liver. Conclusions: During APAP hepatotoxicity, moderate amounts of IL-1β are formed through a caspase 1-dependent mechanism. However, IL-1β is one of many inflammatory mediators produced during APAP overdose and its removal did not impact neutrophil activation, hepatic neutrophil recruitment and liver injury following APAP overdose. Thus, the NALP3 inflammasome and IL-1 signaling mechanisms are not relevant targets for therapeutic interventions in APAP hepatotoxicity.

PS 453 ACETAMINOPHEN HEPATOTOXICITY IN HUMANS: MITOCHONDRIAL INJURY AND DNA FRAGMENTATION IN OVERDOSE PATIENTS.

M. R. McGill[1], M. R. Sharpe[2], C. Williams[1], M. Taha[2] and H. Jaeschke[1]. [1]*Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS and* [2]*Internal Medicine, University of Kansas Medical Center, Kansas City, KS.*

Acetaminophen (APAP) overdose is the leading cause of acute liver failure in the US. In rodents and humans, APAP hepatotoxicity begins with metabolism to the electrophilic intermediate N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI is detoxified by conjugation with glutathione, or it binds to proteins in the cell. In rodents, binding to mitochondrial proteins initiates a cascade of events within mitochondria: oxidative stress, the membrane permeability transition, swelling, lysis, and release of matrix proteins. Endonucleases from mitochondria can then translocate to the nucleus and cause DNA fragmentation. Though well established in mice, these later events have not been investigated in humans. To evaluate mitochondrial injury and DNA fragmentation in the clinic, plasma samples were obtained from APAP overdose patients with injury and from overdose patients without injury as controls. Peak activity of the mitochondrial enzyme glutamate dehydrogenase (GlDH) increased from 0-45 U/L in controls to 132-1699 U/L in plasma from patients with APAP-induced injury, and strongly correlated with alanine aminotransferase (ALT) activity. At the time of peak ALT, mitochondrial DNA (real time PCR) in plasma increased from 0 in controls to as much as 54 ng/mL in patients with injury; in addition, nuclear DNA fragments (ELISA) were as high as 7200% of controls. Mice treated with 300 mg/kg APAP had increases in GlDH activity and nuclear DNA fragments in plasma that were similar to humans. The kinetics of liver necrosis (histology) and TUNEL staining correlated well with plasma ALT and DNA fragments in these animals, indicating that plasma markers accurately reflect tissue injury. Conclusion: These data provide evidence that mitochondrial injury and nuclear DNA fragmentation occur in humans after APAP overdose. Thus, critical steps in the mechanism of APAP hepatotoxicity in humans are similar to what has been shown in mice.

PS 454 KAVA EXTRACT, AN HERBAL ALTERNATIVE FOR ANXIETY RELIEF, POTENTIATES ACETAMINOPHEN-INDUCED CYTOTOXICITY IN RAT HEPATIC CELLS.

X. Yang and W. F. Salminen. *Division of Systems Biology, U.S. FDA, NCTR, Jefferson, AR.*

The widely-used over-the-counter analgesic acetaminophen (APAP) is the leading cause of acute liver failure in the United States and due to this high incidence, a recent FDA Advisory Board recommended lowering the maximum dose of APAP. Kava herbal dietary supplements have been implicated in several human liver failure cases leading to the ban of kava-containing products in several Western countries. In the US, the FDA has issued warnings about the potential adverse effects of kava, but kava DS are still available to consumers. In this study, we tested the potential of kava extract to potentiate APAP-induced hepatocyte cytotoxicity. In rat primary hepatocytes, co-treatment with kava and APAP caused 100% loss of cell viability, while the treatment of kava or APAP alone caused ~50% and ~30% loss of cell viability, respectively. APAP-induced glutathione (GSH) depletion was also potentiated by kava. Co-exposure to kava decreased cellular ATP concentrations, increased the formation of reactive oxygen species, and caused mitochondrial damage as indicated by a decrease in mitochondrial membrane potential. In addition, similar findings were obtained from a cultured rat liver cell line, clone-9. These observations indicate that kava potentiates APAP-induced cytotoxicity by increasing the magnitude of GSH depletion, resulting in oxidative stress and mitochondrial dysfunction, ultimately leading to cell death. These results highlight the potential for drug- dietary supplements interactions even with widely used over-the-counter drugs.

PS 455 HEPATIC ENDOTHELIAL CELLS REGULATE LIVER CELL REPAIR FOLLOWING ACETAMINOPHEN INTOXICATION.

Y. Liu, C. R. Gardner, M. Francis, J. D. Laskin and D. L. Laskin. *Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Acetaminophen (APAP) is a mild analgesic and antipyretic agent known to cause centrilobular hepatic necrosis when taken in excess. Previous studies have demonstrated that hepatic macrophages contribute to the pathogenic process. Whereas early after APAP intoxication macrophages are classically activated to release cytotoxic/ proinflammatory mediators that contribute tissue injury, subsequently they are alternatively activated and release mediators that down regulate inflammation and initiate wound repair. We have discovered that liver endothelial cells (EC)s, like

Kupffer cells, can also be classically and alternatively activated in vitro by inflammatory cytokines upregulated in the liver after APAP administration. In the present studies we determined if these cells play a role in the hepatic response to APAP. ECs were collected from rats 12-72 hr after administration of APAP (600 mg/kg, ip) and analyzed by real time PCR for expression of classical and alternative activation markers. Treatment of rats with APAP resulted in a rapid and transient increase in EC expression of inducible nitric oxide synthase, a marker of classical activation. This was followed at 72 hr by EC expression of arginase I (ArgI), YM2 and FIZZ1, markers of alternative activation. Co-culture of control ECs with hepatocytes pretreated with 5 mM APAP for 2 hr resulted in increased expression of ArgI and mannose receptor demonstrating that APAP-injured hepatocytes release factors important in alternative activation of ECs. APAP administration to rats also resulted in hepatocyte expression of matrix metalloproteinase-9 (MMP9) and connective tissue growth factor (CTGF), markers of tissue repair, which was most prominent after 72 hr. Moreover, control hepatocytes co-cultured with alternatively activated ECs were found to express MMP9 and CTGF. Taken together, these data suggest a role of alternatively activated ECs in liver repair following APAP intoxication. Supported by NIH grants GM034310, ES004738, CA132624, AR055073, ES005022.

PS 456 NEGATIVE REGULATION OF CLASSICAL MACROPHAGE ACTIVATION BY THE STK RECEPTOR DURING ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

C. R. Gardner[1], Y. Liu[1], P. A. Hankey[2], J. D. Laskin[3] and D. L. Laskin[1]. *[1]Rutgers University, Piscataway, NJ, [2]Pennsylvania State University, University Park, PA and [3]UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Classically activated macrophages and mediators they release, including reactive nitrogen species (RNS), play a critical role in the pathogenesis of acetaminophen (APAP)-induced hepatic necrosis. Restricting the activity of these macrophages and production of RNS is key to ameliorating tissue injury and initiating wound healing. Macrophage stimulating protein (MSP) is a liver-derived protein reported to bind to the STK receptor on macrophages resulting in inhibition of NF-κB and down regulation of inducible nitric oxide synthase (iNOS). In the present studies we analyzed the role of MSP signaling via STK in regulating macrophage cytotoxic activity during APAP-induced hepatotoxicity. APAP administration (600 mg/kg, i.p.) to rats resulted in a rapid and transient increase in iNOS mRNA expression in liver macrophages which was evident at 24 hr. At this time, expression of the anti-inflammatory cytokine, IL-10 was suppressed. These are characteristics of classically activated cytotoxic macrophages. Treatment of animals with APAP also resulted in increased expression of STK in liver macrophages. To analyze the role of STK in APAP hepatotoxicity, we used transgenic mice lacking this receptor. We found that STK-/- mice were significantly more sensitive to APAP than wild type mice. Thus while administration of APAP to wild type mice resulted in 8% mortality at 24 hr, in STK-/- mice, mortality was 44%. Moreover, surviving STK-/- mice displayed severe hemorrhagic centrilobular hepatic necrosis with injury extending into the midzonal and portal regions of the liver, which was not evident in wild type mice. Taken together, these findings suggest that MSP signaling via STK is important in down regulating inflammatory responses of cytotoxic hepatic macrophages and promoting repair following APAP intoxication. Supported by NIH GM034310, ES004738, CA132624, AR055073 and ES005022.

PS 457 ROLE OF MACROPHAGE-DERIVED GALECTIN-3 IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

A. Dragomir[1], R. Sun[1], J. D. Laskin[2] and D. L. Laskin[1]. *[1]Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and [2]Environmental and Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Toxic doses of acetaminophen (APAP), a widely used analgesic, are known to cause centrilobular hepatic necrosis. Accumulating evidence suggests that macrophages and inflammatory mediators they release play a key role in the pathogenic process. In the present studies, mechanisms regulating macrophage release of inflammatory mediators were investigated. Galectin-3 (Gal-3) is a beta-galactoside binding lectin secreted by activated macrophages that stimulates production of proinflammatory mediators and reactive oxygen species. Treatment of wild-type mice with APAP (300 mg/kg, i.p.) resulted in a time-dependent increase in Gal-3 expression in the liver, reaching a maximum after 48-72 hr. Immunohistochemistry indicated that Gal-3 was predominantly expressed by macrophages infiltrating into necrotic areas. To analyze the role of Gal-3 in APAP-induced hepatotoxicity, we used Gal-3$^{-/-}$ mice. In wild-type mice, APAP caused a time-dependent increase in serum ALT and AST levels, beginning within 6 hr. This was associated with histological evidence of centrilobular necrosis. These effects were significantly attenuated in Gal-3$^{-/-}$ mice. APAP-induced increases in expression of the proinflammatory cytokines TNF-α, and MCP-1, and lipocalin 2, a marker of oxidative stress, were also reduced in Gal-3$^{-/-}$ mice. In contrast, expression of the antioxidant enzyme, heme oxygenase-1, as well as the anti-inflammatory protein galectin-1, were increased in Gal-3$^{-/-}$ mice relative to wild-type mice following APAP administration. Taken together, these results suggest that Gal-3 plays an important role in macrophage activation and mediator release during the pathogenesis of APAP-induced hepatotoxicity. Supported by NIH grants GM034310, ES004738, CA132624, AR055073 and ES005022.

PS 458 TARGETING OF THIOREDOXIN REDUCTASE BY THE ACETAMINOPHEN METABOLITE N-ACETYL-P-BENZOQUINONE IMINE.

Y. Jan[1], D. E. Heck[2], D. L. Laskin[3], Y. Liu[3], A. Dragomir[3] and J. D. Laskin[1]. *[1]Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, [2]Environmental Health, New York Medical College, Valhalla, NY and [3]Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Acetaminophen (APAP), an analgesic and antipyretic, is bioactivated by cytochrome P450s in hepatocytes to the reactive electrophilic metabolite N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is known to bind to glutathione and a variety of hepatic proteins, an important downstream event mediating acetaminophen hepatotoxicity. Mammalian thioredoxin reductase (TrxR) is a critical cellular antioxidant which contains a selenocysteine in its C-terminal redox center, an accessible target for electrophilic modification. In the present studies, we determined if NAPQI targeted TrxR. APAP (0.5-10 mM) was found to cause a marked inhibition of TrxR activity in primary cultures of rat hepatocytes, and in reaction mixes containing liver microsomes or recombinant P450s and purified rat liver TrxR. To elucidate mechanisms underlying the inhibitory actions of APAP on TrxR, the effects of NAPQI on purified TrxR were characterized. Treatment of TrxR with NAPQI was found to inhibit enzyme activity (IC50 = 23 nM). A recombinant mutant enzyme, in which selenocysteine was replaced with cysteine, was 5000-fold less sensitive to NAPQI, suggesting that selenocysteine was modified. This is supported by our findings that alkylation of TrxR with biotin-conjugated iodoacetamide, which selectively reacts with selenol or thiol groups on proteins, was inhibited by NAPQI. Moreover, LC-MS/MS analysis demonstrated that NAPQI selectively modified the cysteine and selenocysteine residues in the C-terminal redox center of TrxR. Collectively, these data demonstrate that reactive intermediates of APAP can target TrxR and this may be an important mechanism by which APAP overdose induces oxidative stress and cellular damage. Supported by NIH grants GM034310, AR055073, CA093798, CA132624, ES004738, ES005022, and ES017389.

PS 459 TRIFLUOPERAZINE MEDIATED HEPATOPROTECTION AND REDUCTION OF HIF-1α IN ACETAMINOPHEN MEDIATED TOXICITY IN MICE.

S. Chaudhuri[1], S. S. McCullough[1], A. Brown[1], J. A. Hinson[2] and L. P. James[1, 2]. *[1]Pediatrics/Clinical Pharmacology, Arkansas Childrens Hospital Research Institute, Little Rock, AR and [2]Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.*

Mitochondrial permeability transition (MPT) and oxidative stress are proposed mechanisms for APAP mediated hepatotoxicity. Trifluoperazine (TFP) has been previously shown to reduce APAP mediated hepatotoxicity (Yamamoto et al, 1990; Dimova et al., 1995) in vivo. In vitro studies of APAP toxicity in freshly isolated hepatocytes showed that TFP reduced MPT (Reid et al., 2005). We previously reported the induction of HIF-1α in APAP toxicity in mice and have hypothesized that HIF-1α induction is secondary to MPT associated oxidative stress. Thus we examined the effect of TFP on hepatotoxicity and HIF-1α induction in APAP mediated hepatotoxicity. B6C3F1 male mice were pretreated with TFP (10 mg/kg) prior to APAP (200 mg/kg IP). The mice were sacrificed at 1, 4 and 24 h. Mean (± SE) values for ALT were 1786 (± 258) IU/L and 16 (± 2) IU/L for APAP/veh and APAP/TFP mice at 4 h, respectively ($p \leq 0.05$). At 24 h, mean ALT values were 4267 (± 207) IU/L and 3055 (± 928) IU/L for APAP/veh and APAP/TFP mice ($p \leq 0.05$). Histopathological examination of liver sections was consistent with the biochemical data. Hepatic glutathione depletion and hepatic APAP-protein adducts were comparable in both groups of mice at all time points, indicating that TFP did not affect the metabolism of APAP. HIF-1α expression determined by immunoblots showed that HIF-1α expression was reduced by 45% in APAP/TFP mice at 1, 4, and 24 h compared to the APAP/veh mice ($p \leq 0.05$). The data suggest that TFP reduces toxicity in APAP treated mice and that HIF-1α induction is secondary to MPT associated oxidative stress.

PS 460 SULFORAPHANE PREVENTS ACETAMINOPHEN-INDUCED HEPATIC INJURY IN MICE.

R. Schmidt[1, 2], K. C. Falkner[2, 3], J. Beier[1, 2] and G. Arteel[1, 2]. [1]*Pharmacology & Toxicology, University of Louisville, Louisville, KY,* [2]*Alcohol Research Center, University of Louisville, Louisville, KY and* [3]*Medicine, University of Louisville, Louisville, KY.* Sponsor: C. States.

Background. Sulforaphane (SFN), an isothiocyanate found in cruciferous vegetables, is known to confer antioxidant protection in vivo. Rather than directly reacting with free radicals, however, SFN works by inducing Nrf2, a transcription factor that binds to the promoter regions of several known antioxidant genes and enhances detoxification. Because oxidative stress is a major contributor to acetaminophen (APAP)-induced toxicity, SFN may defend the liver against APAP overdose by activating the Nrf2 pathway and increasing endogenous antioxidant response. **Methods.** Six-week-old C57BL/6 mice were acclimated to AIN-76A purified diet, then pre-treated with 10 mg/kg SFN i.g. for four days, and injected with APAP (300 mg/kg i.p.) on the fifth day. Six hours after APAP injection, mice were euthanized with ketamine/xylazine, and their blood and livers were harvested. Liver tissue was snap-frozen in liquid nitrogen or fixed in 10% formalin for sectioning and mounting on microscope slides. Some snap-frozen liver was homogenized in 4.4% metaphosphoric acid for detection of glutathione. **Results.** APAP overdose caused massive spikes in serum liver enzyme activity, indicating hepatic injury. SFN prevented this injury, as evidenced by a 3-fold reduction in ALT activity and a 5-fold reduction in AST. Total liver glutathione was depleted by APAP overdose, but protected by SFN. Finally, histological indices of liver damage were markedly reduced by SFN. Red blood cell infiltration at pericentral regions of the liver was identified with chloroacetate esterase staining, and found to be minimized by SFN treatment. Similarly, 4-hydroxynonenal adducts were seen after APAP overdose, but these adducts were diminished with SFN. **Conclusion.** These data support the hypothesis that SFN attenuates acute APAP-induced liver injury. This decrease in injury results from the increased availability of glutathione to conjugate the reactive metabolites of APAP.

PS 461 ACETAMINOPHEN (APAP) AND S-ADENOSYL-L-METHIONINE (SAME) EFFECTS ON OXIDATIVE STRESS ENZYMES: ATTENUATION BY SAME.

J. Brown, J. G. Ball, T. Ahmad and M. Valentovic. *Pharmacology, Physiology and Toxicology, Marshall University School of Medicine, Huntington, WV.*

Acetaminophen (APAP) is the primary cause of drug induced liver failure in the United States. S-adenosyl-L-methionine (SAMe) participates in the transsulfuration pathway to provide cellular glutathione. SAMe is involved in transmethylation reactions and is critical for growth of liver cells. The transmethylation and transsulfuration pathways are important in recovery from APAP hepatic toxicity. This study tested the hypothesis that SAMe treatment 1 h after APAP overdose would preserve hepatic function by reducing oxidative stress. Male C57BL/6 mice (16-22 grams) were divided into the following groups: vehicle treated with water (VEH; water 15ml/kg ip injections), 250 mg/kg APAP (15 ml/kg, ip) or SAMe (1.25 mmol/kg) which was administered 1 h after APAP injection (SAMe and SAMe+APAP). Hepatic toxicity was evaluated 2 and 4 h after APAP administration. APAP depressed ($p<0.05$) glutathione (GSH) peroxidase and glutathione reductase activity when compared to the VEH group. Glutathione reductase activity was similar between the SAMe+APAP and VEH group. GSH peroxidase activity was higher in the SAMe+APAP group compared to APAP but was lower than the VEH and SAMe treated animals. Total and Mn SOD activity was depressed in the APAP group when compared to the all other groups. Total and Mn SOD activity was comparable between the SAMe+APAP and VEH and SAMe groups. In conclusion, SAMe preserved the activity of antioxidant pathways to reduce oxidative stress induced by APAP overdose. (Supported by NIH Grant 5P20RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence and NIH Grant INBRE 3P20RR016477-09S4).

PS 462 NERVE GROWTH FACTOR (NGF) IN MOUSE LIVER HAS AN AUTOCRINE ACTIVITY AND ITS TRANSCRIPTION IS MODULATED BY BSO, ACETAMINOPHEN, AND ARSENIC.

C. Valdovinos and M. E. Gonsebatt. *Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico.*

Nerve growth factor (NGF) is a member of structurally related proteins, called neurotrophins (NTs), that regulate neuronal survival, development, function, and plasticity. Moreover, NGF in mammals is an important activator of antioxidant mechanisms. These functions of NGF are mediated by tropomyosin-related kinase receptor A (TrkA), which are tyrosin kinases. Although there is evidence that NTs and their receptors are expressed in visceral tissues, the extent to which NTs are involved in their physiology is less clear. There is evidence suggesting that NGF may be involved in lung and skin tissue repair, as well as in allergic inflammation and fibrosis. In the adult mouse NGF is the most expressed liver NT. Although some studies show that NGF is modulated positively in the liver after resection-reperfusion injury as well as after CCl4 injection, there is no information about whether the release of NGF has a paracrine or an autocrine function. Moreover, despite the fact that NGF is an important modulator of antioxidant mechanisms in neural tissues, there are no previous studies relating the oxidative stress and the modulation of NGF in liver. In the present study, we demonstrate that in mouse *ngfb* mRNA levels increase in liver after intraperitoneally injection of 6 mmol/kg L-buthionine-S-R-sulfoximine (BSO), 300 mg/kg acetaminophen (APAP) or 14 mg/kg sodium arsenite. In addition, we show that also the phosphorylation of the NGF high-affinity receptor TrkA in liver is up regulated which suggests an endocrine role of NGF and a probable hepatoprotective effect.

PS 463 URINE PROTEOMIC PROFILING FOR BIOMARKERS OF ACETAMINOPHEN-INDUCED ACUTE LIVER INJURY IN MICE AND HUMANS.

R. P. van Swelm[1], C. M. Laarakkers[2], R. Masereeuw[1] and F. G. Russel[1]. [1]*Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands and* [2]*Laboratory Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.* Sponsor: R. Pieters.

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Introduction: Drug-induced liver injury (DILI) is responsible for most drug withdrawals due to toxicity. Currently no adequate predictive biomarkers are available. Here, urine protein profiling was used to search for early biomarkers of acute liver injury induced by acetaminophen (APAP). Methods: Male FVB mice were given a single ip dose of APAP (0-350 mg/kg bw; n=6-30 per dose). Urine was collected during 24 h and plasma and liver tissue at 24 h after treatment. Human urine samples were collected from patients with acute liver injury caused by APAP-intoxication(n=2), malaria (n=1) and HELLP syndrome (n=1). Urine samples were profiled using MALDI-TOF MS. Differentially expressed proteins were identified using 1D-gelelectrophoresis and MALDI-LTQ and confirmed on Western blot. Results: In mice, APAP doses of 275 and 350 mg/kg bw resulted in significantly elevated plasma alanine transaminase (ALT) values (40-28900 U/L, $p<0.0001$) and centrilobular hepatic necrosis (0-85%, $p<0.0001$), quantitated by blinded histological scoring. Profiling revealed 15 proteins present in urine of mice with APAP-induced liver injury and not in control, including multiple GSTs, carbonic anhydrase 3 and regulcalcin. However, because these proteins only appeared in urine of mice with severe APAP-induced liver injury (ALT > 3000 U/L), they appear not sensitive enough as biomarker. Nevertheless, a mass of 15.9 kDa could be found in urine of relatively mildly affected mice (ALT ~200 U/L) and likely corresponds to superoxide dismutase 1 (SOD1). Western blot analysis confirmed the presence of SOD1 in mouse and human urine, which correlated well with corresponding ALT values ($r=0.996$, $p<0.0001$). Principal conclusion: SOD1 showed to be a sensitive urinary biomarker of APAP-induced liver injury in mice, and has potential to identify patients with acute liver injury.

PS 464 DEVELOPMENT OF A MECHANISTIC SAR MODEL FOR THE DETECTION OF PHOTOTOXIC CHEMICALS AND USE IN AN INTEGRATED TESTING STRATEGY.

S. Ringeissen[1], L. Marrot[1], R. R. Note[1], A. Labarussiat[1], S. Imbert[1], M. Todorov[2], O. Mekenyan[2] and J. Meunier[1]. [1]*L'Oreal, Aulnay Sous Bois, France and* [2]*Laboratory of Mathematical Chemistry, Bourgas, Bulgaria.* Sponsor: H. Toutain.

Phototoxicity is of increasing concern in dermatology, since modern lifestyle is often associated with exposure to sunlight. The most commonly reported process is via oxidative reactions. Therefore characterizing the photo-pro-oxidant potential of a compound early in its industrial development is of utmost interest, especially for compounds likely to undergo sunlight exposure in skin. Today there is a need for filtering compounds to be tested in the 3T3 Neutral Red Uptake in vitro test for phototoxicity since testing requires resources. A computational model aiming at predicting the mechanisms that imply the generation of reactive oxygen species was developed using a diverse set of 56 chemicals having 3T3 NRU data. An historical mechanistic QSAR model developed for polycyclic aromatic hydrocarbons was used to derive the new mechanistic model: descriptors were selected upfront to describe the modeled phenomenon. The historical parabolic relationships between phototoxicity and the energy gap (EGAP) between energies of the Highest Occupied Molecular Orbital and the Lowest Unoccupied Molecular Orbital was confirmed. The model predicts chemicals to be phototoxic or photodegradable, or non phototoxic. A four-step testing strategy is proposed to enable the reduction of experimental testing with the in silico model implemented as a first screen.

PS 465 QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP MODELING OF SKIN SENSITIZERS TESTED IN LOCAL LYMPH NODE ASSAY.

D. Pu[1], H. Zhu[1], G. Zhao[1], J. Strickland[2], E. Salicru[2] and A. Tropsha[1].
[1]University of North Carolina, Chapel Hill, NC and [2]ILS, Research Triangle Park, NC.

Allergic contact dermatitis is caused by skin exposure to reactive small molecules. Animal screening for potential environmental toxicants is time-consuming and costly; therefore, virtual screening using computational models to tag potential sensitizers is an attractive approach to help prioritize chemicals for in vivo evaluation. We have developed QSAR models for a series of small molecules with local lymph node assay (LLNA) data compiled by NICEATM. The dataset was initially curated and harmonized following our standard procedures, and 270 compounds were used to develop QSAR models. We have applied both k nearest neighbor (kNN) and random forest (RF) statistical techniques and Dragon descriptors; all models were generated and validated according to our standard QSAR workflow. A standard 5-fold external cross-validation procedure was employed: the dataset was randomly divided into 5 subsets; each subset was systematically used as an external set while the other four jointly formed a modeling set. The external correct classification rates averaged across 5 validation folds were 76% for both kNN and RF models. The Y-randomization test yielded no statistically significant models, which eliminated the possibility of chance correlation. Model applicability domains (AD) were defined and implemented during both external validation and library screening. The validated models were employed to screen an external chemical library including 607 chemicals which could be skin sensitizers based on indirect evidence (each chemicals has been linked to at least one report in which it tested as a potential skin sensitizer in a certain assay). 242 and 230 chemicals fell within the AD of kNN and RF model, respectively, and 182 (75%) and 175 (76%) of them, respectively, were accurately predicted as skin sensitizers. These results validate the usefulness of QSAR models developed in this study in prioritizing chemicals for in vivo screening as potential skin sensitizers.

PS 466 3D ORGANOTYPIC EPIOCULAR EYE IRRITATION TEST (EIT) RESULTS – USE OF ALTERNATIVE TISSUE CULTURE INSERTS.

M. Klausner, Y. Kaluzhny, P. J. Hayden, L. d'Argembeau-Thornton and H. Kandarova. *MatTek Corp, Ashland, MA.*

Highly differentiated, organotypic tissue models are being used increasingly in lieu of animals to meet regulatory testing requirements. The on-going quality of these tissue models is of prime importance so that US and EU regulators and industry users can be assured that the toxicological system is reproducible both during the validation process and afterwards. The purpose of this study was to investigate the effects of various tissue culture inserts (TCI) on EpiOcular™ tissue morphology and assay reproducibility. 4 types of tissue culture inserts (TCI) from 3 commercial manufacturers were obtained and standardized culture conditions were used to produce the EpiOcular organotypic tissue model, which is compromised of normal human cells. The EpiOcular tissues were subjected to quality control (QC) tests which include histological evaluation and determination of the exposure time of a common surfactant (Triton X-100) that reduces the tissue viability to 50% (ET-50). In addition, the EpiOcular Eye Irritation Test (EIT) was run using 94 test articles (73 liquids, 21 solids). The histology of tissues produced on all 4 types of TCI was structurally equivalent to the control tissues. The average ET-50s for EpiOcular produced were: TCI-1: 23.8 +/- 6.5 min (n=8, p=0.4), TCI-2: 21.3 +/- 5.4 min (n=6, p=0.11), and TCI-3: 26.5 +/- 7.9 min (n=6, p=0.28). These values were not statistically different than that of tissues cultured on the control TCI-C (24.4 +/- 3.7 min). In addition, use of the alternative TCI in the EpiOcular Eye Irritation Test (EIT) showed no differences in irritant/non-irritant predictions for a wide range of chemical categories and irritancies. For TCI-1, EIT sensitivity and specificity were 98.0% and 63.8%; for TCI-2: 96.9% and 67.2%, for TCI-3: 89.9% and 71.7%. Control cultures had specificity and sensitivity of 100.0% and 68.0%. In summary, the TCI is one of the crucial parameters in producing high quality, reproducible, organotypic tissue models, however multiple commercially available TCI appear to have appropriate properties.

PS 467 GUIDING FORMULA DEVELOPMENT UTILIZING THE CLINICAL CUMULATIVE IRRITATION CLINICAL TESTING WHEN INGREDIENTS INTERFERE WITH *IN VITRO* IRRITATION ASSAY.

D. Orak, P. Kazmi, V. Srinivasan and T. A. Re. *Safety Evaluation, L'Oreal USA, Clark, NJ.*

Anti-aging skin care products are formulated with exfoliants, anti-oxidants, moisturizers, skin firming agents, and sunscreens that function to improve the skin's appearance. In our development labs, new formulations containing ascorbic acid, ferulic acid, phloretin, glycols and silicones exhibited unexpected irritation reactions in HRIPT (Human Repeat Insult Patch Test.) The formula and 3 deletion versions (formulae without one or more key ingredients) were tested in an in vitro 3-dimensional skin tissue model to determine the causative irritating agent(s). Only the phloretin deletion formula demonstrated ET50 values correlating with no irritation. Knowing that phloretin itself is non-irritating in other marketed formulations and that anti-oxidants such as ascorbic acid, ferulic acid, and phloretin derivatives have direct reduction effects on MTT or ATP endpoints in the in vitro skin irritation assay, it was decided that other candidate formulas containing phloretin could nevertheless be safely tested in a clinical setting. We selected the 21-day Cumulative Irritation study which utilizes both the degree of irritation as well as the time to irritation as evaluative endpoints as a screen to be able to distinguish between mildly irritating test materials. Six reformulations and 3 reformulations minus key anti-oxidant ingredients were tested and the rank of irritancy was used to give direction to the formulation lab. A revised formulation was subsequently shown to be non-irritating and non-sensitizing in a HRIPT and was demonstrated to have good local tolerance in a dermatologist controlled "in use" study. This work demonstrates how substances with questionable in vitro results can move directly to human testing without the need for further pre-clinical evaluation.

PS 468 A WEIGHT OF EVIDENCE APPROACH TO IMPROVING SAR PREDICTION OF ALLERGIC CONTACT DERMATITIS.

C. Seaman[1], A. Quayson[2], M. J. Olson[3] and F. Guerriero[4]. *[1]HS&P, GlaxoSmithkline, Ware, Herts, United Kingdom, [2]School of Biosciences, University of Birmingham, Birmingham, West Midlands, United Kingdom, [3]HS&P, GlaxoSmithKline, Research Triangle Park, NC and [4]HS&P, GlaxoSmithKline, Philadelphia, PA.*

Assessment of DEREK v10, (Lhasa, UK) and OECD Toolbox v 1.1, (Laboratory of Mathematical Chemistry, Bourgas, Bulgaria) found that their overall accuracy at predicting the sensitizing potential of substances was poor (ca 50 – 56% respectively) for pharmaceutical process chemicals, primarily due to very poor positive predictivity. Negative predictivity was substantially better at 77% and 70% respectively. Using a dataset of 249 diverse chemicals we investigated whether a weight of evidence approach, combining SAR with other available information, such as the historical performance of individual alerts, physico-chemical properties and information on reactivity (positive Ames test) assays would allow for improved predictions. The performance of the more commonly firing alerts within both systems varied widely, with reasonable performance for structures containing halogenated alkane groups (accuracy ca 70% and) and very poor performance for a variety of other alerts. Using physico-chemical properties to predict dermal penetration potential improved overall performance of both systems to ca 70% and 60% respectively. Confirming reactivity with positive Ames results and use of the data-gap filling mode of the OECD Toolbox when appropriate, also aided in improving confidence in predictions of sensitizing potential. In conclusion, consideration of sensitization alerts within a framework of a weight of evidence analysis can aid in interpreting SAR outputs. Further, overall improvements for both systems are identified, including modification of the more poorly performing alerts such as those for aldehyde precursors (accuracy 27%), or ketones (accuracy 14%).

PS 469 A MECHANISTIC RATIONALE FOR THE PREDICTION OF SKIN IRRITANCY EFFECTS IMPLEMENTED IN A WORKFLOW PROCESS.

C. Yang[2], S. Ringeissen[1], J. F. Rathman[2, 3], B. Prunier[1], R. Note[1] and J. Meunier[1]. *[1]L'Oreal Recherche, Aulany-sous-Bois, 93600, France, [2]Altamira LLC, Columbus, OH and [3]The Ohio State University, Columbus, OH.*

As part of the integrated assessment strategies, a decision tree algorithm was developed to predict a molecule's potential for skin irritancy (SI) severity effects based on in vivo Draize legacy data (OECD TG404). While developing alternatives to animal testing, it indeed makes sense to leverage existing in vivo data by integrating such knowledge with the computational toxicology effort.The algorithm was developed for diverse chemical classes using a training set of chemicals representative of the cosmetic industry. It adopts the combination of rulebase and regression models. Regression models are based on a set of structural features and physicochemical descriptors selected on the basis of the underlying mechanisms of action (e.g. bioavailability, chemical reactivity, interactions with constituents of cell membrane). For example, since surfactants are essential ingredients in cosmetic formulations due to their capacity to form micelles in solution, special emphasis is on measuring and calculating properties associated with nonionic surfactants containing polyethoxylated-chains, compounds that may induce SI effects via interactions with the phospholipid components of cell membranes. One representative structural rule is based on the ratio of EO-chains to alkyl chain length, i.e. hy-

drophilic/lipophilic balance. Other descriptors include the extent to which these molecules partition into lipids, headgroup surface area to volume ratio, and molecular shape. Experiments with model phospholipid membranes were devised to study mechanisms of interaction and consequently derive new descriptors. Overall this predictive scheme improves the reliability of SI estimations. Further, to make it sustainable within the safety assessment process and easily accessible with user-friendly features for toxicologists, the decision tree is being implemented in a pipeline workflow as part of skin knowledgebase.

PS 470 DECISION TREES FOR EVALUATING SKIN AND RESPIRATORY SENSITIZING POTENTIAL OF CHEMICALS FOR REACH.

M. Selgrade[1], K. Sullivan[1], R. Boyles[1], E. Dederick[1], T. Serex[2] and S. Loveless[2]. *[1]ICF International, Fairfax, VA and [2]DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE.*

REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), in addition to other regulatory and product stewardship programs, requires the assessment of chemicals for skin and respiratory sensitizing potential. Because manufacturers and importers are required to gather information on the properties of chemicals for registration in a standardized format, scientists around the world will be involved in such assessments. Guidance for determining the sensitizing potential of chemicals is available in EC Regulation No. 1272/2008 Classification, Labeling, and Packaging of Substances (CLP); REACH guidance from the European Chemicals Agency (ECHA); and the United Nations Globally Harmonized System (GHS). To assess the sensitizing potential of chemicals, we created three decision trees for evaluating skin and respiratory sensitizers and chemical mixtures. Our approach 1) brings all the regulatory information into one brief document that provides a transparent, step-by-step method to evaluate the evidence that individual chemicals or mixtures have sensitizing potential; 2) provides an efficient, uniform approach that promotes consistency when evaluations are done by different reviewers; and 3) provides a standard way to convey the rationale and information used to classify chemicals. We applied this approach to >50 chemicals (that varied with respect to the amount of testing data available) distributed among 11 evaluators. Evaluators had varying degrees of expertise and were identified as primary or secondary reviewers. Evaluators found the decision trees easy to use and the end recipients of the sensitization analyses found the resulting documentation to be consistent across users and very useful. Application and evaluation of our approach allows for transparency, process management (e.g., documentation, change management and version control), as well as consistency in chemical hazard assessment for REACH and other programs. Further validation of this approach is desirable.

PS 471 DEVELOPMENT OF A MICROBIAL RISK ASSESSMENT GUIDELINE.

M. The Federal Interagency. *USDA, U.S. EPA, DHHS and other Federal Agencies, Washington, DC.* Sponsor: A. Kadry.

Microbial risk assessment (MRA) approaches are rapidly evolving to inform decision-making for control of microbial pathogen-related health risks. The United States Environmental Protection Agency (EPA) and Department of Agriculture (USDA), in cooperation with other Federal agencies, are working to leverage their resources, experience, and expertise to generate tools, methods, and guidelines for performing an MRA. The purpose of this interagency activity is to develop guidance on common approaches, where feasible and appropriate, for risk assessors to perform an MRA. The goal is to produce a flexible set of tools, methods, and approaches that can be selected for use in various situations. While there are similarities to chemical risk assessment, microbial risks involve some unique considerations such as secondary transmission, growth, and fluctuations in microbial populations. In recognition of the needs and mandates of different agencies and the various statutory authorities that may apply to MRA, the Guideline emphasizes the need for a flexible template for conducting an MRA. The present draft Guideline is broadly focused on infectious diseases associated with the gastrointestinal tract and fecal/oral transmission in mainly food and water. Future efforts could focus on biothreat agents, airborne microbial hazards, and agriculturally or industrially important microorganisms to supplement this Guideline. The main components of microbial risk assessment (planning and scoping, hazard identification, hazard characterization, exposure, and risk characterization), including its unique considerations, will be outlined in the presentation with points concerning risk management and communication from a risk assessor's perspective.
(The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA., USDA,DHHS and other Federal agencies)
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PS 472 PRELIMINARY SENSITIVITY EVALUATION FOR CUMULATIVE IMPACTS ANALYSIS.

G. V. Alexeeff, J. Faust, L. Meehan and L. Zeise. *OEHHA, California/EPA, Oakland, CA.*

Established risk assessment methods are limited in their capacity to evaluate risks faced by communities with exposures to a range of chemical hazards via multiple routes, and with varying susceptibility and vulnerability among residents. The standard risk assessment approach was originally developed primarily to identify "safe" levels for a single contaminant or to quantify the risk of individual pollutants in a single medium (e.g., tolerances for pesticides in food; standards for contaminants in drinking water; limits on carcinogens such as benzene in air emissions). These assessments often allow for differing susceptibility, but within narrow ranges, for example, individual-level intrinsic biological factors, such as genetic differences leading to pharmacokinetic variability. A growing body of literature suggests that cumulative factors of multiple exposures, susceptibility, and vulnerability may be inadequately addressed in the current risk assessment process. We developed a method to screen for community-scale cumulative impacts. The method uses a concept of total pollution burden based on measures of exposure, public health effects and environmental effects. This is modified by measures of sensitive populations and relevant socio-economic factors, analogous to a relative risk model. We explored the method by applying it to 28 California communities and using publicly available measures of exposures, effects, sensitivity and vulnerability. Sensitivity analysis considered the model structure and the interdependence of parameters. None of the measures was significantly correlated with one another. Multiplicative and additive models gave comparable results. Data gaps identified with the model include the need for community level data to better describe sensitive individuals.

PS 473 CATEGORICAL REGRESSION: STRENGTHS, WEAKNESSES, AND APPLICATIONS.

L. T. Haber[1], R. Danzeisen[2] and J. S. Gift[3]. *[1]Toxicology Excellence for Risk Assessment (TERA), Cincinnati, OH, [2]International Copper Association, New York, NY and [3]U.S. EPA ORD NCEA, Research Triangle Park, NC.*

Categorical regression is a dose-response modeling approach that applies regression analysis to response data in the form of ordered categories of severity. It has been applied in risk assessment for improving duration extrapolation for acute exposures, for calculating risk above the RfD, and for integrating diverse data sets into an understanding of the dose-duration-severity of response. However, this method is not yet used broadly by the risk assessment community, perhaps because of insufficient familiarity with the method. This presentation presents the strengths, weaknesses and some applications of categorical regression, to help address this issue. One key strength is the meta-analytical potential, in which quantal, continuous, and descriptive/categorical data can be combined for a more complete description of the chemical's effects. A corresponding consideration is the need for careful evaluation of the implications of combining data types and endpoints, depending on the issue being addressed. A weakness is that information can be lost in doing the categorization (e.g., variance for continuous measures). In calculating the risk above the RfD, a strength of categorical regression is that it can consider the full spectrum of potential responses and the shapes of the respective dose-response curves. This means that the modeling can account for the potential for different endpoints to be more sensitive in different parts of the dose-response curve. A final consideration is that categorizing the data for input to the modeling can be labor intensive. Scientific judgment is also involved, but this judgment is not substantially different from that involved in identifying NOAELs/LOAELs, or in judging severity of response for such applications as the acute exposure guideline level (AEGL) program. Results are compared with those of benchmark dose (BMD) modeling, where possible.

PS 474 USE OF POWER LAW MODELING TO ESTIMATE LOW DOSE HUMAN RELATIVE POTENCIES OF DIOXIN-LIKE COMPOUNDS (DLCS).

T. B. Starr[1], E. A. Carlson[3], C. H. Sutter[2], T. R. Sutter[2] and J. B. Silkworth[3]. *[1]TBS Associates, Raleigh, NC, [2]University of Memphis, Memphis, TN and [3]General Electric, Niskayuna, NY.*

Donor-to-donor variation in human cell cultures provides a challenge for dose response modeling. This is particularly evident when estimating relative potencies (REPs) for DLCs. In this study, large inter-donor variation in the maximal agonist effect for CYP1A1 induction was seen in normal human epidermal keratinocyte (NHEK) cultures exposed separately to aryl hydrocarbon receptor (AHR) agonists 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,6,7,8-hexchlorodibenzo-p-furan (HxCDF), and 3,3',4,4',5-pentachlorinated biphenyl (PCB 126). Estimation

of REPs required a complex mixed-effects Hill model that assumed DLC partial agonism. The accuracy of 50% effective concentration (EC50)-derived REPs in the presence of uncertain maximal effects of differing magnitudes is questionable. However, the responses observed at low agonist concentrations appeared to be extremely consistent across NHEK donor cultures. To capitalize on this feature, responses and concentrations were first log10-transformed, revealing a donor-invariant dose at which a minimally significant response (MSR) was first achieved. After removal of high concentration data at which the responses showed evidence of saturation, a simple linear regression model of log relative response against log concentration (a power law model) was used to directly estimate the "minimally significant" dose (MSD), using only data from the remaining doses at which statistically significant responses were observed. The resulting MSDs and the related low-dose REPs were largely invariant, regardless of whether or not the response slopes (Hill coefficients) were assumed equal across the different AHR agonists. By simultaneously modeling all three compounds, low-dose REPs were derived. Overall, this power law modeling procedure for REP derivation can likely be applied to in vitro systems where donor variability in response and/or the presence of partial agonists may pose problems for EC50 estimation.

PS 475 NON-THRESHOLD BIOLOGICAL PROCESSES AND THE ASSUMPTION OF LOW-DOSE LINEARITY: CONSIDERATION OF RECEPTOR-MEDIATED EVENTS IN RISK ASSESSMENT.

P. D. White, M. A. Spassova, R. P. Subramaniam and L. Kopylev. *U.S. EPA, Washington, DC.* Sponsor: S. Vulimiri.

Important classes of toxic substances act through biological processes that modify the function of nuclear receptors. The shape of dose-response relationships for these processes continues to be contentious. A brief review is presented of scientific arguments for non-threshold and for low-dose linear behavior and their relevance for receptor-mediated toxicity. Dose-response inferences based upon mechanistic arguments, dose-additivity to background biological processes, and the impact of population variability are discussed. Secondly, a focused review and statistical analysis of examples of empirical data on dose-response relationships for receptor mediated biological responses is presented. Misimpressions regarding the shape of such relationships due to plotting of observed data on semi-log plots are elucidated. For example, a low-dose linear curve, such as a Michaelis-Menten relationship, will appear "sigmoidal" when plotted on a semi-log scale, yet one can easily see that a Michaelis-Menten relationship is linear at low doses when the plots are presented on linear scales. Datasets illustrating receptor mediated effects including induction of P450 enzymes, epidermal growth factor receptor regulation, and observed damage to DNA are statistically analyzed. The data analyzed are generally consistent with low-dose linearity, (e.g., are acceptably fit using Michaelis-Menten relationships) and generally do not show evidence of steep (non-proportional) decrease within the studied dose ranges. In specific cases where steeper than linear dose-response relationships are observed for apical toxicological responses at high dose, limits on the magnitude of a low-dose linear component of the risk relationship are examined.
Disclaimers: The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

PS 476 THE HUMAN-RELEVANT-POTENCY-THRESHOLD: UNCERTAINTY ANALYSIS AND HUMAN CALIBRATION FOR CUMULATIVE RISK ASSESSMENTS.

E. Sargent[2], R. Golden[4], D. Dietrich[3], G. Casella[5] and C. Borgert[1]. [1]*Applied Pharmacology & Toxicology, Inc., Gainesville, FL,* [2]*EV Sargent LLC, Clearwater, FL,* [3]*Faculty of Biology, University of Konstanz, Konstanz, Germany,* [4]*ToxLogic, Potomac, MD and* [5]*Department of Statistics, University of Florida, Gainesville, FL.*

Cumulative effect models based on concentration addition (CA), e.g., relative potency, TEF/TEQ, Hazard Index, are the defaults for toxicological health risk assessments of chemical mixtures with constituents presumed to have similar modes of action or to produce similar toxic effects. CA models predict toxic effects according to the relative potencies of mixture constituents summed by dose, extrapolating the prediction irrespective of the potency of the chemicals or the concentrations to which humans may be exposed. Methods to assess uncertainties inherent in this extrapolation are seldom employed and methods for calibrating the extrapolation to potencies and concentrations relevant for humans have yet to be devised. Using anti-androgens (phthalates, procymidone, finasteride, DES) as a test dataset, we demonstrate that uncertainties in cumulative effects data for the observable toxicity range in rodent studies may be 2-fold or more, and may be orders of magnitude greater when extrapolated to lower, human-relevant doses. We present a novel Human-Relevant-Potency-Threshold approach that can improve application of the concentration addition model and obviate a need to use default uncertainty factors in deriving human-protective point-of-departure doses (e.g., RfDs) for estimating cumulative toxicity and projecting human risk. The HRPT approach uses data for hormonally active pharmaceutical chemicals or natural receptor ligands, prioritizing human clinical data and relating the human observable effect range to those from animal toxicology studies. We discuss how an HRPT for anti-androgens might be developed and how it could be used to improve the application of CA models in cumulative risk extrapolation.

PS 477 THRESHOLD ANALYSES FOR ACRYLONITRILE AND BRAIN TUMORS IN RATS.

D. E. Strother[1] and C. R. Kirman[2]. [1]*ToxSolve LLC, Manassas, VA and* [2]*Summit Toxicology LLP, Orange, OH.*

The potential risks from exposures to acrylonitrile (AN) have historically been assessed based upon its potential carcinogenicity, primarily driven by brain tumor response in rats. Analyses were performed to evaluate the presence of a threshold for brain tumor response in rats based upon pooled data, which includes twelve data sets (six oral and six inhalation), spanning 34 nonzero dose groups and 12 control groups, expressed in terms of an internal dose measure (peak 2-cyanoethylene oxide or CEO in brain). Stepwise addition of the nonzero dose groups indicates no evidence of a dose-response relationship below 0.012 mg/L (peak CEO in brain). The slope of the dose-response relationship above 0.012 mg/L is significantly different from zero, and is significantly different from the slope of the dose-response relationship predicted below 0.012 mg/L. Inclusion of a threshold term did not result in a significant improvement of the fit of a Weibull dose-response model to the pooled rat brain tumor data, demonstrating the difficulty in proving the existence of a threshold based solely upon changes in goodness of fit tests. The dose-response relationship for AN and rat brain tumors is well correlated with published measures for oxidative stress (8-oxo-dG, oxidative DNA damage), while showing a lack of correlation with direct DNA damage. Together the statistical and biological approaches provide support for adopting a nonlinear dose-response assessment (i.e., cancer reference values) when assessing risks to human populations exposed to AN.

PS 478 EFFICIENT DESIGN OF BIOLOGICAL EXPERIMENTS FOR DOSE-RESPONSE MODELING IN TOXICOLOGY STUDIES.

F. Yang[1] and D. Porter[2]. [1]*West Virginia University, Morgantown, WV and* [2]*NIOSH, Morgantown, WV.*

This work is concerned with the efficient design of biological experiments to quantify the dose-response relationship of a substance, which is one of the most fundamental steps in risk assessment. To obtain such relationships, biological experiments need to be performed at different dose levels to observe the corresponding bioactivity responses of animals. Because of costs, ethics, or other limitations, sample sizes are usually restricted and efficient use of resources is critical. Thus, the design of experiments, i.e., the selection of experimental doses and the allocation of animals, plays an important role in the estimation of dose-response relationships. Efficient design for dose-response modeling is challenging due to the special features of toxicity data, i.e., the possibly nonlinear nature of dose-response curves and the typical variance heterogeneity (non-constant variance) involved. In this work, an experimental design procedure particularly suitable to toxicity data collection is developed to guide the dose selection and animal allocation in experiments. The design procedure is built in a two-stage Bayesian paradigm, which provides a statistically valid mechanism to utilize prior information for the design of future experiments. Most appropriate dose-response and variance models are identified to describe the toxicity data. Bootstrapping, a computationally intensive resampling method as opposed to conventional statistical inference methods, are used to derive important information from the preliminary data required by the subsequent experimental design. To achieve practically useful designs, multiple design criteria are considered simultaneously, and multi-objective metaheuristics are adapted to search for a set of Pareto optimum designs, which allow for the evaluation of various trade-offs in practical experimental settings. The efficiency of the proposed design methods over conventional designs is demonstrated through extensive simulation experiments as well as the toxicology study of a series of engineering nanomaterials.

PS 479 PHARMACOKINETIC AND TOXICOLOGICAL PROFILING OF NATURAL PRODUCTS AND DRUGS.

C. Hasselgren[1], H. Chen[2], Y. Yang[2], S. Boyer[1], T. I. Oprea[3] and S. Muresan[2]. [1]*Global Safety Assessment, AstraZeneca, Mölndal, Sweden,* [2]*DECS, AstraZeneca, Mölndal, Sweden and* [3]*Division of Biocomputing, University of New Mexico, Albuquerque, NM.*

Natural products are often considered relevant starting points in drug discovery projects due to their diversity, novelty and intrinsic biological activity. In addition to being complex structures and difficult to synthesize, a major challenge when working with libraries based on scaffolds derived from natural products is their

pharmacokinetic and toxicological profile optimization. During the development of a new lead compound, several in silico tools and models are normally used. These are however built using compounds that may look quite different from conventional drug compounds and/or natural products and have a different distribution in their physicochemical properties. To understand how chemistry and pharmacokinetic and toxicological profiles compare between natural products and synthetic molecules and to determine the reliability of our current tools, we have analyzed and compared a number of data sets from different sources. These were the Clinical candidate database (9 800 compounds) and the Natural Product database (121 600 compounds) from GVKBio, a set of 2 000 oral drugs and the KEGG set of 11 700 compounds. For all datasets, we computed physicochemical properties as well as standard DMPK and safety models such as Caco-2, Ames and hERG. We reviewed the structural space overlap between the different sets using Principal Component Analysis (PCA) and established usability of our current models for the natural drug compounds. Physicochemical property models can be validated against experimental data but safety models can mainly be assessed by space coverage and model domain applicability as experimental data is not generally available for natural products. When possible, validation against experimental data was done. The results show that there are some significant differences between the distributions of simple molecular properties and that the classes (synthetic drug/natural drug) can be partially separated using PCA. The reliability of our current models is also discussed in relation to these differences.

PS 480 A FLEXIBLE METHOD FOR BUILDING AND USING PREDICTIVE MODELS APPLIED TO SAFETY ENDPOINTS.

O. Spjuth[2], L. Carlsson[1], M. Eklund[2], E. Ahlberg Helgee[1] and S. Boyer[1]. *[1]Computational Toxicology, AstraZeneca, Mölndal, Sweden and [2]Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden.*

We present a general method for building and using predictive in silico models. The method can take any collection of compounds with activities as input, and automatically produces several different predictive models; (1) Similarity searches using InChI, signatures and fingerprints (exact matches and nearest neighbours) (2) A classification or regression QSAR model using a support vector machine (SVM) and interpretable signature descriptors (3) Discriminative signatures (structural alerts or toxicophores). All models can be run simultaneously for novel chemical structures and produce interpretable results with important substructures highlighted in a chemical editor or spreadsheet, with the possibility to generate hypothesis on how to avoid safety hazards by making changes to the chemical structure and watch how this change affects the predictions. The method is available from the Bioclipse workbench, can be run on desktop computers and does not necessarily require a network connection. We demonstrate our method on publicly available data for three endpoints: mutagenicity, carcinogenicity, and AhR inhibition. The results indicate that combining local data with established and well tested models together with interpretable results can aid scientists in making better informed decisions in safety assessment.

PS 481 ASSESSING DOSE-RESPONSE OF ENVIRONMENTAL CHEMICALS INTERACTION WITH DNA USING A SYSTEM BIOLOGY MODELING APPROACH.

J. M. Gavina, Y. Feng and A. Nong. *Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada.*

Genotoxicity of environmental pollutants is investigated at the molecular level for DNA damage or instability, disruption of cellular metabolic processes, carcinogenicity, or mutagenic modifications. Chemical interaction with cellular DNA can be screened using genetic or proteomic assays, sensitive chromatography, or mass spectrometry techniques. The difficulty with the in-vitro chemical screens at the target receptor lies in the interpretation of these results for in-vivo or even human internal dose levels. System biology modeling approaches integrate genomic and proteomic data with biological mechanism of response. These models provide understanding of the process as well for correlation in doses ranges of concern. In this pilot study, a biological model or system motif was developed to described the kinetic reaction of DNA adduct formation following exposure to different chemicals (tetrachlorohydroquinone, styrene-7,8-oxide, methyl methane sulfonate, PGE, and BPDE). A HPLC screening method was used to detect and quantify DNA products. A simple experiment was designed to expose double stranded DNA oligonucleotides under controlled conditions over time and various doses. DNA adducts were then separated and measured respectively by chromatography and spectrometry. The motif model parameters were then calibrated with the time series and validated with the dose-response relationship data. The estimated rate constants of these reactions are chemical dependent. The dose-response behaviour is linear, where a Hill coefficient of 1 was estimated, for all chemicals except for tetrachlorohydroquinone. This in-vitro system motif provides as basis for more complex biological toxic response such as cellular DNA damage. Combined with internal dosimetry models (e.g. PBPK), system biology models can serve as a useful tools to predict the dose-response at the target receptor for environmental exposure risk assessment of contaminants.

PS 482 USING A MULTIPLE STAGE DECISION TREE TO MAKE ACTIVITY CALLS IN QUANTITATIVE HIGH-THROUGHPUT SCREENING (QHTS) DATA.

K. R. Shockley[1], G. E. Kissling[1], R. Huang[2], M. Xia[2], C. P. Austin[2] and R. R. Tice[1]. *[1]National Toxicology Program/National Inistitute of Environmental Health Sciences, Research Triangle Park, NC and [2]NIH Chemical Genomic Center, Bethesda, MD.*

The goals of the Tox21 collaboration are to identify mechanisms of toxicity, prioritize chemicals for in vivo testing and predict adverse responses to environmental chemicals in humans. Quantitative high throughput screening (qHTS) assays provide an opportunity to meet these goals, holding the potential for wide chemical coverage, reduced cost of testing on a per-substance basis and minimal use of animals. Moreover, the ability of a substance to induce a toxicological response is better understood by analyzing the response profile over a broad dose range than by evaluating effects at one or a few doses. Pharmaceutical applications of qHTS generally seek to minimize false positives in activity assessment and often rely on heuristic algorithms to make activity calls. In contrast, multiple questions must be asked to determine the toxicological relevance of a chemical and statistical testing should be used to minimize false negatives. Here, we developed a multiple-stage decision tree statistical model and applied it to qHTS data from nine cell-based nuclear receptor agonist assays (AR, ER, FXR, GR, PPARα, PPARλ, RXR, TRβ, VDR). Data were fit to a four-parameter Hill equation and an overall F-test comparing the best fit to the Hill equation and a horizontal line (no response) was calculated for each chemical. Substances with a robust dose-response were identified in the first stage. In the second stage, compounds not detected as active in the first stage were evaluated for a maximal response at the lowest dose by comparing the distribution of measured responses to a control value. Chemicals with a weak dose-response were identified in the third stage, and the final stage separated substances exhibiting a cytotoxic response at the lowest dose from inactive compounds. Our model identified more active compounds than a previously utilized heuristic approach.

PS 483 TESTING MULTIPLE AVAILABLE QSARS FOR REPRODUCTIVE TOXICITY AND CARCINOGENICITY TO DRINKING WATER CONTAMINANTS.

M. Heringa[1], A. Roncaglioni[2], R. Benigni[3] and A. Worth[4]. *[1]KWR Watercycle Research Institute, Nieuwegein, Netherlands, [2]Istituto di Ricerche Farmacologiche "Mario Negri"(IRFMN), Milan, Italy, [3]Istituto Superiore di Sanita (ISS), Rome, Italy and [4]European Commission - Joint Research Centre (JRC), Ispra, Italy.*

Within the REACH legislation in Europe, Quantitative or Qualitative Structure Activity Relationship- (QSAR-) toxicity predictions are an important alternative to animal testing. Also in the hazard identification of detected drinking water contaminants for which no experimental toxicity data can be found, QSARs are helpful. However, proper use of QSARs is not always straightforward, as there are many models available, each with its own specifications. To illustrate what a risk-assessor runs into when using QSARs, toxicity predictions were sought for 10 selected case compounds for the endpoints mutagenicity, carcinogenicity, and reproductive toxicity (here: developmental toxicity and estrogenicity). The models applied were: CASETOX, TOPKAT, Derek for Windows, PharmaAlgorithms, the OSIRIS IRFMN RBA model, HazardExpert, Toxtree, and lazar. Only two of these models could be judged as "scientifically valid", a requirement posed by REACH, which is based on the five OECD principles. The other models were used as supportive information. For mutagenicity, for eight of the ten compounds all models (seven) agreed in their predictions, for carcinogenicity this was the case for one compound only, for developmental toxicity for three compounds and for estrogenicity for nine compounds. The application of multiple QSAR models has made apparent that it is unclear how conflicting predictions of multiple models should be evaluated. This indicates a strategy on the use of multiple QSAR models is necessary, for which some ideas are discussed.

PS 484 CONSENSUS MULTIPLE-POTENCY QSAR MODELING FOR PREDICTION OF RODENT CARCINOGENICITY.

E. J. Matthews[2] and K. P. Cross[1]. *[1]Leadscope, Inc., Columbus, OH and [2]U.S. FDA/CFSAN/OFAS, College Park, MD.* Sponsor: R. Tice.

The performance of quantitative structure-activity relationship (QSAR) models depends extensively upon the quality of the data used to construct the models, and the methods employed to optimize model predictive performance. For rodent carcinogenicity models, the nature, location, prevalence, and extensiveness of tumors

arising during the bioassay all affect the overall test call, the ratio of actives and inactives affect model performance. However, the specific criteria for assigning positive calls varies for agencies having different regulatory statutes, and different levels of concern are applied to chemicals having caused isolated single site tumors versus trans-gender, trans-species, and multiple site tumor responses. Furthermore, one investigator may require a high sensitivity model to predict as many carcinogens as possible while another may require a high specificity model to avoid predicting noncarcinogens as carcinogenic. This study used FDA toxicology data and Leadscope software to develop a new, multi-potency, QSAR methodology to satisfy investigators with different needs. Each compound having tumor findings was scored as a low, medium, or highly potent carcinogen in the male and female, rat and mouse, test conditions. Next three QSAR models were constructed per endpoint: 1) a low carcinogenic potency model where low, medium, and high potency carcinogens are all scored as positive; 2) a medium potency model where medium and high potency carcinogens are scored as positive; and 3) a high-potency model where only high potency carcinogens are scored as positive. Run independently, each model can address a specific investigator's need regarding carcinogenic potency. Run together, the 3 potency models permit consensus modeling of the results that achieves both high sensitivity and high specificity.

PS 485 DECISION ANALYSIS APPROACH TO MODE-OF-ACTION MODELS: EXPLICIT EXPRESSION OF DATA QUALITY AND RELIABILITY TO EVALUATE CAUSALITY.

A. M. Jarabek[1, 2] and D. Crawford-Brown[2]. *[1]U.S. EPA, Research Triangle Park, NC and [2]DESE UNC, Chapel Hill, NC.*

Recent assessment approaches use weight of evidence (WOE) and human relevance frameworks to evaluate evidence on the mode of action (MOA) for a chemical, promoting transparent consideration of key events of pathogenesis for specific endpoints. To date, what is often lacking is an explicit expression of data quality, utility, and reliability to support claims of causality for a given key event or set of events embodied in an MOA or biologically-based dose-response model; important because judgments concerning data on individual parameters for specific steps influence the confidence in the ultimate decision regarding causality. We propose a two-step decision analytic (DA) process to evaluate causality. The 1st step is to populate an MOA model with specific data to support the development of parameters for key events. Description of the pathogenesis process is divided into characterization of two key components: dosimetry (toxicokinetics) and tissue response (toxicodynamics). The 2nd step entails evaluating both the ability of the data to describe or represent the particular parameter or process, and the extrapolation premises or assumptions required to apply these data to the human disease target context. This requires explicit evaluation of the data from various observational contexts: in vitro, in vivo laboratory animal and human studies. Data are assigned to evidence categories (direct empirical, semi-empirical, empirical correlation or theory-based inference) to assess relevance. Coherence of data within an observational context and then across contexts is used to arrive at a summary judgment of causality for each of the two key characterizations. These are combined to arrive at overall confidence in the conclusion regarding the causal role of the proposed key event(s). The DA approach aids data integration across different domains pertinent to a decision on MOA, facilitates formal inferences, and is amenable to both quantitative and qualitative applications. (These views are the authors and do not represent U.S. EPA policy).

PS 486 CASE STUDIES ON THE ROLE OF DNA ADDUCT DATA IN CANCER RISK ASSESSMENT: CONTEXT IS KEY.

J. Skare[1], L. H. Pottenger[2], L. Andrews[2], A. Bachman[3], P. J. Boogaard[4], J. Cadet[5], P. Farmer[6], M. Himmelstein[7], A. Jarabek[8], J. Kim[9], E. Martin[10], R. Mauthe[11], R. Persaud[12], J. Preston[8], R. Schoeny[8], J. Swenberg[13], G. Williams[14], F. Zhang[2] and E. Zeiger[15]. *[1]Procter & Gamble, Cincinnati, OH, [2]The Dow Chemical Company, Midland, MI, [3]EMBSI, Annandale, NJ, [4]Shell International, The Hague, Netherlands, [5]CEA/Grenoble, Grenoble, France, [6]U. Leicester, Leicester, United Kingdom, [7]DuPont, Newark, DE, [8]U.S. EPA, Research Triangle Park, NC, [9]HESI, Washington, DC, [10]AstraZeneca, Macclesfield, United Kingdom, [11]Pfizer, Groton, CT, [12]L'Oreal, Clark, NJ, [13]UNC, Chapel Hill, NC, [14]NY Medical College, Valhalla, NY and [15]E. Zeiger Consulting, Chapel Hill, NC.*

The biological significance and role of DNA adduct data in risk assessment are debated. An ILSI/HESI committee published an approach for the evaluation of DNA adduct data in a key event dose-response framework for a mutagenic mode of action (MOA) analysis for cancer risk (Jarabek et al., 2009). The approach stresses the need to create a context for adduct data in conjunction with other key types of data, such as dosimetry, mechanistic response data, and tumor incidence. The committee has applied this systematic approach to three case studies (Aflatoxin B1, tamoxifen, vinyl chloride). Specific challenges to data synthesis for these chemicals include: background or endogenous adduct levels, different key events in rodents versus humans, and data quality and reliability. Analysis of the case studies led to general principles for evaluating the role of adduct data in MOA: target tissue and adduct type depend on exposure concentration, duration, and internal dose determinants such as physico-chemical properties, anatomical and physiological factors, and ADME processes; adduct profiles can change with duration or dose, due to differences in repair/persistence of specific adducts. Characterization, with structural identification, is necessary for adduct use in MOA assessment. Key conclusions include: DNA adduct data cannot be used in isolation to determine a mutagenic MOA, and DNA adducts represent biomarkers of exposure and not of effect. DNA adduct data alone are informative but not sufficient to assign a mutagenic MOA. (These authors' views do not represent USEPA policy).

PS 487 QSAR PREDICTION OF CARCINOGENIC POTENCY (TD50) AND THE RISK SPECIFIC DOSE(RSD) ADJUSTED THRESHOLD OF TOXICOLOGICAL CONCERN (TTC) FOR GENOTOXIC CHEMICALS AND PHARMACEUTICAL IMPURITIES.

J. F. Contrera. *Computational Toxicology Services LLC, Rockville, MD.*

The Threshold of Toxicological Concern (TTC) is a level of exposure to a genotoxic impurity that is considered to represent a negligible risk to humans. The TTC was derived from the results of rodent carcinogenicity TD50 values that are a measure of carcinogenic potency. The TTC currently sets a default limit of 1.5 μg/day in food contact substances and pharmaceuticals for all genotoxic impurities without carcinogenicity data that does not take carcinogenic potency into account. Bercu et al. (Regul. Toxicol. Pharmacol. 2010, 57: 300-306) proposed a risk specific dose (RSD) that is a carcinogenic potency adjusted TTC for genotoxic impurities based upon a QSAR predicted TD50. This promising approach is currently limited by the software used, a combination of MC4PC (www.multicase.com) and an Eli Lilly and company in-house software (VISDOM) that is not available to the public. In this report the TD50 was predicted using a commercially available software, SciQSAR (formally MDL-QSAR, www.scimatics.com) employing the same TD50 training data set and external validation test set that was used by Bercu et al. (2010). The predicted TD50 was then used to calculate the RSD.The results demonstrate the improved performance of SciQSAR for predicting TD50 values and the general applicability of QSAR predicted TD50 values to determine the RSD for genotoxic impurities. Using the RSD, an acceptable level for a genotoxic impurity can be determined based on compound specific QSAR calculated carcinogenic potency rather than the default worst case TTC limit.

PS 488 VIRTUAL CHEMICAL-PROTEIN RECEPTOR INTERACTIONS CAN DIFFERENTIATE TUMOR SITE SELECTIVE CARCINOGENS.

N. Malik, S. Qamar, C. Carrasquer and A. R. Cunningham. *James Graham Brown Cancer Center, University of Louisville, Louisville, KY.*

Structure-activity relationship models for carcinogenesis typically use descriptors that are chemical descriptors and develop models based on chemicals that are classified as carcinogens or non-carcinogens. Here we report the use of the cat-SAR expert system using biological descriptors (i.e., chemical-receptor interactions) for selective tumor site rat carcinogen sets (clitoral gland, esophagus, hematopoietic system, kidney, large and small intestine, liver, lung, mammary gland, nasal cavity, nervous system, and uterus). One group, the Target-Site Carcinogens – Non-Carcinogens (TSC-NC), was composed of active chemicals that were carcinogenic to the target site and inactive chemicals that were whole animal non-carcinogens. The second group, the Target Site Carcinogens – Non-Target Site Carcinogens (TSC-NTSC), was composed of active chemicals that again were carcinogenic to the target site but the inactive category was composed of carcinogens to any/all other sites except the target site. The novel SAR biological descriptors were derived from the virtual screening for ligand-receptor interactions of carcinogens and non-carcinogens to a set of 5494 proteins. Cross-validation of triplicate TSC-NC models resulted in the esophagus model with an average concordance value of 90%, three models with average values in the 80%s (clitoral gland, nasal cavity, and nervous system), five in the 70%s (kidney, lung, mammary gland, small intestine, and uterus), and three in the 60%s. The TSC-NTSC triplicate models returned three models with average concordance values in the 80%s (esophagus, small intestine, uterus), seven in the 70%s (clitoral gland, hematopoietic system, large intestine, lung, mammary gland, nasal cavity, and nervous system), and two in the 60%s. We speculate that using biological descriptors for SAR models, can enhance model predictivity, help bridge the gap between chemical exposure and potential carcinogenesis, and identify novel molecular targets anticancer drug discovery.

PS 489 USE OF 'OMICS DATA TO IMPROVE PAH MIXTURES CANCER RISK ASSESSMENT.

P. McClure[1], H. Carlson-Lynch[1], J. Stickney[1], K. Salinas[1], I. Cote[2] and L. Flower[2]. [1]*Chemical, Biological, and Environmental Center, SRC, Inc., North Syracuse, NY and* [2]*Office of Research and Development, U.S. EPA, Washington, DC.*

Cancer risks from PAH mixtures are estimated using slope factors for whole mixtures (e.g., coke oven emissions) or, when whole-mixture slope factors are not available, an approach using relative potency factors (RPFs) and a slope factor for benzo[a]pyrene (BaP). Environmental degradation and compositional variance of PAH mixtures are key sources of uncertainty in whole-mixture approaches. In the RPF approach, uncertainty is compounded by the lack of cancer data for many PAHs. Efforts to decrease uncertainty in these approaches with more rodent bioassay data are hampered by several issues including high expense. Because omic technologies hold promise as alternatives or supplements to rodent bioassays, we analyzed data from recently published in vitro studies comparing gene expression and post-transcriptional changes from carcinogenic and non-carcinogenic PAHs or PAHs of differing genotoxic or carcinogenic potencies. In human MCF-7 and HepG2 cells, carcinogenic PAHs [e.g., BaP, dibenzo[a,l]pyrene (DBalP)] modulated many more genes than noncarcinogenic PAHs and a correspondence was apparent between genotoxic and carcinogenic potency and the total number of genes modulated [DBalP > BaP > benzo[b]fluoranthene > fluoranthene (FA)]. PAHs with the highest potency (e.g., DBalP, BaP) modulated a number of genes in the p53 signaling pathway, while noncarcinogenic or low potency PAHs (benzo[e]pyrene, FA) modulated no genes in this pathway. Studies of proteins in HepG2 cells exposed to PAHs or PAH-containing soil extracts provide supporting evidence that consideration of post-transcriptional changes of gene products in the mdm2-p53 network may enhance the prediction of carcinogenic potential of PAHs or PAH mixtures. The analysis provides impetus for further research to develop omics-based testing schemes to predict the carcinogenic potential of untested PAHs or PAH mixtures. [The views expressed in this abstract are those of the authors and do not represent the policy of the US EPA.]

PS 490 SOME TRICKS FOR STATISTICAL ANALYSIS OF NEOPLASTIC LESIONS USING PROC MULTTEST IN SAS.

A. K. Thakur and H. Chen. *Nonclinical Safety Assessment, Covance Laboratories, Vienna, VA.*

There are two approaches to statistical evaluation of neoplastic lesions for a carcinogenicity study- regression techniques using variations of logistic distribution and interval based or the so-called IARC stratified method. Because of simplicity of the method and the ease of incorporating exact permutation techniques, the interval based method has gained popularity among investigators. Various regulatory agencies also prefer this latter method. The statistical analysis package SAS provides a procedure called the MULTTEST, which is capable of performing both asymptotic and exact tests for neoplastic lesions incorporating cause of death information. The tests are both for association or trend as well as heterogeneity where one compares the treated group incidences versus control. There are several issues regarding this procedure under MULTTEST. Irrespective of whether one is interested in asymptotic or exact trend probability, if one wishes to use the nominal dose levels which are often unequally spaced, the trend probability one gets is actually the one based on ordinal or equally spaced dose levels unless one uses the "CONTRAST" statement specifying the actual dose levels. Also, if the nominal dose levels are not integers, then the procedure reverts back to equally spaced or ordinal dose levels irrespective of whether one uses the "CONTRAST" statement. In this latter case, to get the correct result for trend, one has to integerize the dose levels (such as changing a dose of 0.01 mg/kg/day to 10 μg/kg/day by multiplying by 100). This conversion with the "CONTRAST" statement as before will produce the correct trend probability. Integerizing the dose levels does not change the strength of the trend. The heterogeneity probabilities are not affected. We will provide examples and show how to proceed appropriately.

PS 491 A GROUP APPROACH FOR HUMAN HEALTH SAFETY ASSESSMENT OF FRAGRANCE INGREDIENTS.

A. Api, S. Bhatia, C. Letizia, D. McGinty, J. Scognamiglio and L. W. Smith. *Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

While chemical structure assessments have been used for some time (Hall,1968, Ford,2000, Bickers,2003) the safety evaluation of fragrance ingredients presents unique challenges. Over 2500 chemically defined ingredients in commerce, many used at low levels, were classified into 92 parent structural groups based on chemical reactivity. Subsequent subdivisions of major groups were established using the same principles in order to create structure-activity groups of reasonable size. Some generalizations are possible: 1) 88% are structurally simple, low molecular weight, predominantly semi-volatile substances consisting of carbon, hydrogen and oxygen, 2) The majority can be assigned to homologous groups with predicted consistency of metabolism and toxicity, 3) For most groups, detoxication yields innocuous metabolites, 4) Systemic exposure levels are typically below thresholds of toxicological concern. A primary assumption is that the structural homologies allow safety issues to be considered for several materials within the context of information which exists for the structural group as a whole, eg, reading across among individual materials within the group. In many cases existing information for a structural group may obviate the need to submit a particular individual substance to full toxicological testing. In other cases, it may be necessary to test one or more members of a structural class to obtain more robust data to solidify assessment of the class. To date, 10 groups of fragrance ingredients have been evaluated and published, the most current being the "Alcohols Branched Chain Saturated" group. Currently 27 groups are in preparation: 6 of these are in final review, 11 are in data analysis and 10 are in data collection and preliminary assessment.

PS 492 IMPLEMENTING COMPUTATIONAL METHODS IN AN INSTITUTIONAL KNOWLEDGE-BASE AT FDA'S CENTER FOR FOOD SAFETY AND APPLIED NUTRITION: A MODE-OF-ACTION-BASED APPROACH TO BUILDING QSAR MODELS.

L. Ye, R. Brown, E. Busta, K. Arvidson and C. Yang. *CFSAN, U.S. FDA, College Park, MD.*

The Chemical Evaluation and Risk Estimation System (CERES) project under development at FDA emphasizes providing reviewers with easy-to-use decision support tools for their safety reviews. As part of the knowledge-base, a series of structural rules and computational models are being developed and implemented. At FDA CFSAN, a team of toxicologists and chemists provides input and feedback on computational methods development including the QSAR models. The CERES project adopted a philosophy of transparent training sets and mode-of-action(MoA)-driven models for the development of Quantitative Structure Activity Relationship (QSAR) models. In this poster, salmonella reverse mutation and in vitro chromosome aberration endpoints are discussed. These assays are accepted by FDA Redbook and ICH guidelines for evaluating potential mutagenicity and clastogenicity, respectively, and further interpreted for potential carcinogenicity. For salmonella mutation, models were built for classes where chemical reactivity and metabolic transformations play a role. For chromosomal aberrations, structural classes that can result in direct DNA damage or interrupt DNA replication were added to reflect mechanisms. In addition, the experimental conditions of cell types (CHL or CHO cell lines) and treatment method (pulsed or continuous) were modeled separately. The individual MoA models were then pulled together to give a final overall weight-of-evidence result. The training sets are compiled from public databases and publications as well as FDA CFSAN and CDER data. The QC process of the dataset preparation and the rules to resolve conflicts from different data sources will be discussed along with the prediction performance.

PS 493 ASSESSMENT OF MITOCHONDRIAL TOXICITY OF ENVIRONMENTAL CHEMICALS USING A QUANTITATIVE HIGH-THROUGHPUT SCREENING APPROACH.

M. S. Attene-Ramos[1], R. Huang[1], S. Sakamuru[1], S. Shahane[1], L. Shou[1], K. L. Witt[2], R. R. Tice[2], C. P. Austin[1] and M. Xia[1]. [1]*National Institutes of Health Chemical Genomics Center (NCGC), Rockville, MD and* [2]*National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.*

As part of the U.S. Tox21 initiative, the NCGC is developing and optimizing cell-based and biochemical assays suitable for quantitative high throughput screening (qHTS) in a 1536-well format. This effort will generate pathway profiles for environmental compounds that will facilitate the evaluation of mechanisms of toxicity and prioritization for more extensive testing, as well as the development of predictive models for in vivo toxicity. In this study, we optimized a mitochondrial membrane potential assay using the water-soluble JC-10 sensor to detect mitochondrial depolarization in HepG2 cells and we then used this method to evaluate the mitochondrial toxicity of 1408 environmental compounds provided by the NTP. In response to mitochondrial depolarization, the ratio of the cytosolic green fluorescent monomeric form to the mitochondrial red fluorescent aggregate form increases. Of the 1408 compounds screened over a 14-point concentration curve (0.5 nM to 92 μM), 44 compounds disrupted the mitochondrial potential in HepG2 cells after treatment for one hour. We selected 33 compounds for further studies, including

high-content imaging. Thirty-two compounds were confirmed in both fluorescence plate reader and imaging assay formats. To study the structure-activity relationship of these mitochondrial disruptors, we clustered these compounds by structural similarity. This analysis resulted in four structural clusters and 15 singletons. These clusters may be useful for identifying structural features associated with mitochondrial toxicity. Our results confirm the robustness of this assay for identifying mitochondrial membrane-potential disruptors in qHTS format. Supported by NIEHS Interagency Agreement Y2-ES-7020-01.

PS 494 QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) MODELING OF ESTROGEN RECEPTOR (ER) BINDING AFFINITY AND VIRTUAL SCREENING FOR POTENTIAL ENDOCRINE DISRUPTING COMPOUNDS (EDCS).

L. Zhang[1], H. Zhu[1], A. Afantitis[2], G. Melagraki[2], H. Sarimveis[2], I. Rusyn[3] and A. Tropsha[1]. *[1]Eshelman School of Pharmacy, UNC, Chapel Hill, NC, [2]NovaMechanics Ltd., Nicosia, Cyprus and [3]Environmental Sciences and Engineering, UNC, Chapel Hill, NC.*

Regulatory agencies, both in Europe and in the US, require substantial, and costly, animal testing of EDCs. The use of computational predictors to prioritize potential candidates for in vivo endocrine disruption tests could significantly reduce the cost of experiments and the number of compounds subject to testing. In this study, we have employed combinatorial QSAR modeling workflow, incorporating rigorous model validation procedures (Tropsha, Mol. Inf., 2010, 29, 476-488), to the largest publicly available dataset of ER binding compounds. The dataset containing 436 ERα and 113 ERβ ligands was compiled from public sources and carefully curated. Each compound was characterized by chemical descriptors calculated with Dragon, CDK, and Mold2 Software. To evaluate the true predictivity of models, 5-fold external cross validation procedure was applied. For each modeling set including 80% of all compounds, k-nearest neighbors (kNN) QSAR approach and each of the three descriptor sets were used for model development. The external predictive accuracy of models ranged between R2 values of 0.63-0.80 and 0.66-0.85 for ERα and ERβ models, respectively. Y-randomization test yielded no statistically significant models, indicating the robustness of our models. The consensus models generated by combining predictions from all independent models passing both training and test set accuracy thresholds had the highest external accuracy, characterized by R2 values of 0.72 and 0.76 for ERα and ERβ models, respectively. We have applied our models for virtual screening of several chemical libraries of interest including the 30K compounds registered under REACH as well as EPA-10K compound set. Approximately 3,000 compounds were identified and prioritized as potential EDCs for future in vivo endocrine disruption tests.

PS 495 A COLLABORATIVE PROJECT FOR DATA SHARING AND HARMONIZATION OF DEVELOPMENTAL TOXICITY DATABASES.

E. Busta[1], S. Espada[2], M. Martin[3], D. Hristozov[1], K. Arvidson[1], D. Mattison[4] and C. Yang[1]. *[1]U.S. FDA, College Park, MD, [2]Pennsylvania State University, College Station, PA, [3]U.S. EPA, Research Triangle Park, NC and [4]NICHD, Bethesda, MD.*

Developmental toxicity is one of the most important non-cancer endpoints for both environmental and human health. Despite the fact that numerous developmental studies are conducted as required for regulatory decisions, there are not yet sufficient data available to develop predictive computational methods. The Food and Drug Administration's (FDA) Center for Food Safety and Applied Nutrition (CFSAN) has been involved in developing ToxML (a toxicity database model) and toxicity databases. The National Center for Computational Toxicology (NCCT) team at Environmental Protection Agency (EPA) implemented ToxRefDB with its data model inspired by ToxML. The new initiative at FDA CFSAN to build an institutional knowledge-base, the CERES (Chemical Evaluation and Risk Estimation System) project, has imposed the need to generate another database model for the Center's regulatory data. The prospect of creating yet another toxicity data model was a real concern; hence, as part of the CERES project, an experiment was conducted at FDA CFSAN to evaluate EPA's ToxRefDB data entry tool for capturing developmental effects from FDA's approved drugs. In collaboration with National Institute of Child Health and Human Development (NICHD), we have added developmental data on 80 more drugs and merged with the existing database of 720 chemicals from EPA and FDA. By incorporating pharmaceutical data into the current agrochemical domain, both the chemical space and biological profiles were expanded. This experiment also assisted our database modeling while expanding and harmonizing the controlled vocabulary. It is our further goal to combine the data with existing developmental toxicity databases to support computational method development. This poster will present comparisons of developmental toxicity data models, profiles of distinct and common effects, and the chemical domains in both ToxRefDB and CERES. This abstract does not necessarily reflect policies of US EPA, US FDA, and NICHD.

PS 496 DEHP: COMPARISON OF *IN SILICO* PREDICTIONS WITH *IN VITRO* AND *IN VIVO* TOXICITY.

M. M. Dingemans[1], E. Rorije[2], A. Efremenko[3], H. Clewell[3] and B. Blaauboer[1]. *[1]Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands, [2]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands and [3]The Hamner Institutes, Research Triangle Park, NC.*

This research was conducted within a larger program to investigate possibilities and limitations of the use of non-animal data for predicting human risk. In the part described here, the *in silico* predicted effects for bis(2-ethylhexyl) phthalate (DEHP; using knowledge-based systems DEREK and OECD Toolbox, and QSAR-based TOPKAT®) are compared with those identified *in vitro* and *in vivo*, to investigate whether *in vitro* studies can confirm predicted effects.
Toxic effects by DEHP are predicted to be teratogenicity and testicular toxicity (DEREK), carcinogenicity and developmental toxicity (TOPKAT, DEHP in training set) and DNA binding (metabolism required; OECD Toolbox). In particular endocrine disruption, reproductive toxicity (including testicular toxicity) and hepatotoxicity by peroxisome proliferation, and non-genotoxic carcinogenicity are identified *in vitro*. The predicted DNA binding is not confirmed *in vitro*, possibly due to a lack of metabolism. *In vivo* toxicity data for rodents after oral exposure were obtained from North American and European risk assessment agencies, revealing increased liver weight and testicular toxicity as the most critical effects (<10 mg/kg bw in chronic studies). Increased liver weight in rodents is probably related to peroxisome proliferation, which is commonly assumed to be not relevant for humans. The most critical toxic *in vivo* effect of DEHP (testicular toxicity) is also predicted *in silico* and confirmed *in vitro*. However, several toxic endpoints are identified only *in vitro*, while their *in vivo* relevance remains unclear. As metabolism is critical for the *in vivo* toxicity of DEHP, we consider it essential that prediction of metabolite formation is included. By identifying tissue concentrations of chemicals and active metabolites by kinetic modeling, the most relevant toxic endpoints identified *in vitro* can be selected, while avoiding animal testing.

PS 497 CHEMICALS IN CONTEXT: EPA DESIGN FOR THE ENVIRONMENT GREEN CHEMISTRY EVALUATIONS FOR SAFER CONSUMER PRODUCTS.

C. Baier-Anderson[1], C. Davies[1], D. DiFiore[1], K. Grant[1], D. Johnson[2], E. Lavoie[1], L. Morlacci[2], E. Sommer[1] and M. Vrabel[1]. *[1]Design for the Environment Branch, U.S. EPA, Washington, DC and [2]SRC, Inc., Arlington, VA.*

Background: Formulated consumer products are a source of consumer and environmental exposures to chemical hazards. With the number and variety of formulated products on the market, and current limitations of cumulative risk assessment methods, one path to risk reduction is through hazard reduction. The challenge is to identify safer substitutes that function on a par with the more hazardous chemicals currently in use. Approach: Chemicals are classified by their functional use in products. Solvent, chelating agent, surfactant and fragrance are examples of functional ingredient classes. Each chemical is evaluated for human health and ecological hazard endpoints. Available data on the chemical and analogs, and estimations from computer models are assembled, creating a hazard dossier for each chemical. The data are then compared with EPA Design for the Environment (DfE) criteria, which are based on UN Globally Harmonized System and US EPA New Chemicals Program (TSCA) approaches to characterizing chemicals. Results: Chemical safety and preferability are visualized as a continuum within each functional ingredient class, ranging from "Of concern" (red) to "Improved" (yellow) to "Safer" (green). Chemicals that meet or exceed all criteria are considered safer and therefore preferable. Chemicals that fail one or more criteria fall further back on the continuum. The continuum shifts towards the safer end of the spectrum as the state of green chemistry advances. Several examples of chemicals in context are provided, illustrating the spectrum of possible results, and the challenges of decision-making with incomplete and conflicting data. Conclusion: Application of DfE criteria helps identify safer chemicals within ingredient classes and has encouraged reformulation in many of the 2000+ DfE-labeled consumer and institutional products, and prevented 500 million pounds of pollution.

PS 498 APPLICATION OF AGE-SPECIFIC ADJUSTMENTS TO CANCER POTENCY FOR CHILDREN AT CALIFORNIA SCHOOL SITES.

S. A. Knadle[1], H. Bolstad[2], M. S. Sandy[1], M. A. Marty[1] and J. Carlisle[1]. *[1]Cal/EPA, OEHHA, Sacramento, CA and [2]Environmental Toxicology, University of California at Davis, Davis, CA.*

Legislation to protect children's health in California arose when a massive learning center was unknowingly built on land containing volatile hazardous waste. Health & Safety Code 900 and 901 mandated OEHHA to provide safe health guidance values for early life exposure to contaminants and to publish guidance for assessing child-specific routes of exposure unique to the school environment. Other legislation requires investigation of proposed school sites for contaminants and risk assessment of any found. OEHHA has presented individually and lists here the oral noncancer child specific reference doses (chRDs). OEHHA also evaluated the influence of age at exposure on potency, analyzing data from studies in which groups of animals are exposed to a carcinogen during different lifestages. A policy was subsequently adopted to weight cancer risk by a factor of 10 for exposures from the third trimester up to age 2, and a factor of 3 for exposures from age 2 up to 16 years. The default Age Sensitivity Factors (ASFs) account for age-specific sensitivity and the longer time for cancer to manifest after early life exposure. Here OEHHA presents application of the default ASFs, in conjunction with age-appropriate exposure factors, to produce child-specific cancer risk estimates (chCRs) for carcinogens. In addition OEHHA presents a detailed risk calculation for chlordane, which is frequently present at schools over the safe level. It illustrates the need for ASFs in estimating risk from early life carcinogen exposure and deriving chCRs that are protective of children. Without ASFs, school staff with a 25-year exposure duration will show a higher risk than physiologically more sensitive children. With ASFs, a daycare/preschool child with 5 years exposure and a 1st – 12th grade student each accrue twice the cancer risk as an adult working at the school for 25 years. This poster will provide the details to assess child risk at California school sites using the mandated guidance and exposure spreadsheet on the OEHHA website.

PS 499 DERMAL DNEL SETTING: USING QSAR PREDICTIONS FOR DERMAL ABSORPTION FOR A REFINED ROUTE-TO-ROUTE EXTRAPOLATION.

V. Mostert and A. Goergens. *Toxicology/Human Exposure, Dr. Knoell Consult GmbH, Leverkusen, Germany.*

The dermal route is one of the most significant routes of exposure to industrial chemicals. However, for the majority of these chemicals, only oral NOAELs are available as point of departure for human risk assessment. As a result, route-to-route extrapolation has to be conducted when setting exposure limits for the dermal route. When calculating Derived-No-Effect Levels (DNELs) for the dermal route under REACh, it is a default assumption that dermal absorption is 100%. Hence, the dermal DNEL will be as low as the oral DNEL. Experimental data on dermal absorption of industrial chemicals is rather scarce, so that the DNELs obtained by route-to-route extrapolation are bound to be overly conservative. To overcome this, we propose a banding approach for the percentage of dermally absorbed dose. This approach is based on the QSAR proposed by Guy and Potts (*Pharmaceutical Research* **9**(5), 663-9, 1992). The underlying formula requires input of molecular weight and octanol-water partition coefficient. In addition, water solubility of the substance is used as conservative surrogate parameter for the concentration of the substance on skin to arrive at flux values (mg/cm^2/h). The following bands of flux values (I) are proposed for dermal absorption (% absorbed per 8-h shift): $I < 0.001$ mg/cm^2/h: 10% absorption; $0.001 \leq I < 0.005$ mg/cm^2/h: 20% absorption; $0.005 \leq I < 0.05$ mg/cm^2/h: 50% absorption; and $I \geq 0.05$ mg/cm^2/h: 100% absorption. These arbitrarily assigned bands were compared with measured absorption data for a number of chemicals, spanning a range of chemicals in terms of water solubility, polarity and molecule size. The comparison showed that in most cases the banding approach will overestimate dermal absorption. As a consequence, dermal DNEL setting will still be conservative, but in many cases a significant refinement of a DNEL extrapolated from an oral point of departure will be possible.

PS 500 AN ASSESSMENT OF THE IMPACT OF EXPOSURE DURATION AND INTENSITY ON THE HUMAN KINETIC ADJUSTMENT FACTOR (HKAF).

K. Krishnan[1] and M. Valcke[1, 2]. *[1]Santé environnementale et Santé au travail, Université de Montréal, Montréal, QC, Canada and [2]Direction de la Santé environnementale et de la Toxicologie, Institut national de santé publique du Québec, Montréal, QC, Canada.*

The HKAF is intended to replace the default value of 3.16, based on chemical-specific kinetic data or models. HKAFs for acute exposure scenarios have not been evaluated. The objective of this study was to evaluate the impact of the duration and intensity of exposures on the HKAF. A PBPK model was used for computing the dose metrics (i.e., area under the blood concentration vs time curve (AUC), maximum blood concentration (Cmax) and the amount metabolized/L liver/24 hr (MET)) in adults, neonates (0-30 d), children (1-3 yrs) and pregnant women (PW) following inhalation exposure to benzene (BNZ), styrene (STR), and 1,1,1-trichloroethane (TRI), for four durations (i.e. 10 min, 60 min, 8 hrs, 24 hrs) and concentrations. For each chemical, the concentrations chosen were based on the chemical's RfC (low) and on six of U.S. EPA's AEGLs (high). Distributions for body weight (BW), height (H) and hepatic CYP2E1 content were obtained from the literature or from P3M software whereas body surface area, blood flows and tissue volumes were calculated from BW and H. The magnitude of HKAF was calculated as the ratio of the 95th percentile value of DM in sensitive subpopulation to the 50th percentile value in adults, obtained by Monte Carlo simulations. Based on AUC and Cmax, the greatest HKAF was observed in neonates regardless of the exposure scenario considered. At low levels of exposure, ranges of AUC- or Cmax-based HKAF were 1.13 – 3.42 depending on the chemical, with STR presenting the highest values. At high levels of exposure, this range was 1.45 – 2.33 for BZ, 3.38 – 5.06 for STR and 1.12 – 1.15 for TRI. On the basis of MET, HKAF were the highest in PW for BNZ (1.48 – 1.7) and varied between 1.72 and 1.86 for STR and 1.98 and 2.12 for TRI. Overall, these results have demonstrated for the first time that the chemical-specific HKAF varies as a function of exposure duration and intensity of inhalation exposures.

PS 501 EVALUATION OF THE IMPACT OF DEMOGRAPHY ON THE ADEQUACY OF THE HUMAN KINETIC ADJUSTMENT FACTOR (HKAF).

M. Valcke[1, 2] and K. Krishnan[1]. *[1]Santé environnementale et Santé au travail, Université de Montréal, Montréal, QC, Canada and [2]Direction de la Santé environnementale et de la Toxicologie, Institut national de santé publique du Québec, Montréal, QC, Canada.*

The HKAF, aimed at replacing the default value of 3.16 to account for population variability in toxicokinetics, is likely to be influenced by the characteristics of a substance and the population. The objective of this work was to evaluate the impact of demography on the numerical value of HKAF. Monte-Carlo simulations were applied to a steady-state algorithm to generate distributions of blood concentration (Cblood) and rate of metabolism (RAM) for oral and inhalation exposure to chloroform (TCM), styrene (STR) and 1,4-dioxane (1,4-D) in three populations composed of various proportions of adults, elderly, children, neonates and pregnant women (PW). Subgroup-specific input parameters were obtained from the literature or P3M software. HKAF was computed as the ratio [99th percentile/median] of dose metrics for the entire population. For oral exposure, Cblood-based HKAF considering U.S. demography varied between 1.46 (TCM) and 2.78 (1,4-D), whereas those based on RAM varied from 1.43 to 1.58. Comparable values were obtained for the inhalation route. In all cases, HKAF varied by less than 2% in the three hypothetical populations considered. Furthermore, the results suggest that the Cblood-based HKAF would cover at least 95% of the individuals in all subgroups except for oral exposure to 1,4-D in neonates (85%) as well as for inhalation exposure to all 3 chemicals in neonates (46 - 85%), to 1,4-D in toddlers (92%) and to TCM and STR in PW (93%). The results also suggest that RAM-based HKAF would cover 83 - 87% of PW for the inhalation of all 3 chemicals. Overall, this study has for the first time evaluated the adequacy of HKAF for protecting the most sensitive individuals of populations with different demographic characteristics.

PS 502 DEVELOPMENT OF SURROGATE REFERENCE CONCENTRATIONS FOR VOLATILE ORGANIC COMPOUNDS COMMONLY ENCOUNTERED AT HAZARDOUS WASTE SITES.

K. Fehling[1], T. Copeland[3], J. Otani[4] and B. Kerger[2]. *[1]The Fehling Group, LLC, Novato, CA, [2]Health Science Resource Integration, Inc., Tallahassee, FL, [3]Consultant, Agoura Hills, CA and [4]Consultant, Menlo Park, CA.*

The USEPA has derived inhalation reference doses (RfDs) and reference concentrations (RfC) for many volatile organic compounds (VOCs) to quantify potential noncarcinogenic human health hazards in risk assessment. For VOCs that do not have an inhalation RfD or RfC, previous convention was to extrapolate the oral reference dose to an inhalation reference dose. While this practice was tenuous from a toxicological standpoint, data gaps necessitated such an approach to assess human health hazards for VOCs encountered at hazardous waste sites. In 2009, the USEPA revised the methodology used to quantify potential health hazards to VOCs. Specifically, the inhaled dose was no longer calculated, rather, VOC concentrations in air were quantified for the potentially exposed populations. This effectively eliminated the ability to use inhalation RfDs and those extrapolated from

oral RfDs, thereby limiting VOC inhalation hazard index calculations to only those with RfCs derived from inhalation studies. This has reduced the number of VOCs that may be quantitatively addressed from well over 100 to approximately 50. The Nevada Division of Environmental Protection (NDEP), in an effort to expand the quantification of health hazards from VOCs, has derived surrogate RfCs for 41 additional chemicals. These surrogates are based upon structure activity relationships and similar toxic endpoints. These surrogates were used to derive Basic Comparison Levels or NDEP screening levels. While these toxicological surrogates are not intended to support cleanup actions, they do provide a conservative (e.g., health-protective) approach to focusing the assessment on those VOCs that may present the greatest threat to human health. Should a surrogate-based chemical be driving the health hazards, then NDEP recommends that a more in depth evaluation of that chemical's toxicity be conducted. The surrogates and their derivation are provided in this presentation.

PS 503 EFFECTS OF AN ASYMMETRY PARAMETER ON CURVE-FITTING FOR SINGLE CHEMICAL AND MIXTURE CONCENTRATION-RESPONSE DATA: 3) MONO-HALOGENATED ACETONITRILES AND ETHYL ACETATES.

D. A. Dawson[1], G. Pöch[2] and T. W. Schultz[3]. [1]*Biology/Toxicology, Ashland University, Ashland, OH,* [2]*Pharmacology and Toxicology, University of Graz, Graz, Austria and* [3]*Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN.* Sponsor: D. Fort.

Research has noted inclusion of an asymmetry parameter can improve curve-fitting of concentration-response data. This approach was applied to several mixture toxicity data sets and compared with the previous method. Microtox data from seven duplicated concentrations and controls for each single agent (i.e., A, B) and mixture (A:B) at three exposure duration (15, 30, 45-min) had been collected previously. Combinations containing mono-halogenated acetonitriles (XANs – X = halogen), ethyl acetates (EXACs), or both (XAN:EXAC) were tested. The data were fit to sigmoid curves using the four-parameter logistic function (4PL) and the five-parameter logistic minus one-parameter (5PL-1P) function. For the 4PL, parameters included minimum effect (0.01 < min < 1.0), maximum effect (max < 100), EC50 and slope, while for 5PL-1P the minimum effect parameter was removed and an asymmetry parameter (s) was added (0.1 < s < 10). The 5PL-1P also used the maximum effect constraint (max < 100). In all cases, mean r2 values were higher for the 5PL-1P than the 4PL [e.g., XAN:EXAC – 5PL-1P = 0.9987, 4PL = 0.9978 (n = 81)]. The 5PL-1P curves had r2 values greater than (89%) or equal to (8%) those for the 4PL for 288 of 297 curves. Agents with high asymmetry values showed greater differences in r2 values and calculated EC50 values were often sufficiently different to affect calculation of predicted dose-addition and independence curves. This, on occasion, affected the type of combined effect observed. The results of this study support inclusion of an asymmetry parameter for curve-fitting of single agent and mixture concentration-response data.

PS 504 EFFECTS OF AN ASYMMETRY PARAMETER ON CURVE-FITTING FOR SINGLE CHEMICAL AND MIXTURE CONCENTRATION-RESPONSE DATA: 2) TWENTY RANDOMLY SELECTED SOFT ELECTROPHILE-CONTAINING MIXTURES.

N. Genco[1], H. Bensinger[1], Z. Il'Giovine[1], D. A. Dawson[1], T. W. Schultz[2] and G. Pöch[3]. [1]*Biology/Toxicology, Ashland University, Ashland, OH,* [2]*Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN and* [3]*Pharmacology and Toxicology, University of Graz, Graz, Austria.* Sponsor: D. Fort.

Recent mixture toxicity studies with soft-electrophiles have examined potential relationships between relative reactivity and time-dependent toxicity of the agents and the combined effects of their binary mixtures. In these studies, toxicity was assessed at 15, 30 and 45 minutes of exposure with seven concentrations and a control (each duplicated) for each single agent and mixture. Concentration-response data were fit to sigmoid curves using the four-parameter logistic function (4PL) and the five-parameter logistic minus one-parameter (5PL-1P) function. For the 4PL, parameters included minimum effect (0.01 < min < 1.0), maximum effect (max < 100), EC50 and slope, while for the 5PL-1P the minimum effect parameter was removed and an asymmetry parameter (s) was added (0.1 < s < 10). The 5PL-1P also had the maximum effect constraint (max < 100). Concentration-response data obtained from twenty combinations of Michael acceptors, SN2-H-polar agent or nonreactive agents were randomly selected for comparison of the curve-fitting functions. The r2 values for 5PL-1P curves were greater than (54%) or the same as (30%) those for 4PL curves for 152 of 180 curves. Calculated EC50 values were sometimes fairly different between functions; thereby affecting calculation of predicted dose-addition and independence curves and, on occasion, the interpretation of combined effect. By comparing the degree of asymmetry among the agents singly it was observed that with increasing asymmetry there was a greater disparity in calculated parameters between the 5PL-1P and 4PL functions, accounting for the improved curve-fitting with 5PL-1P.

PS 505 EFFECTS OF AN ASYMMETRY PARAMETER ON CURVE-FITTING FOR SINGLE CHEMICAL AND MIXTURE CONCENTRATION-RESPONSE DATA: 1) MONO-, DI- AND TRI-HALOGENATED ACETONITRILE COMBINATIONS.

D. Guinn[1], D. A. Dawson[1], T. W. Schultz[2] and G. Pöch[3]. [1]*Biology/Toxicology, Ashland University, Ashland, OH,* [2]*Comparative Medicine, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN and* [3]*Pharmacology and Toxicology, University of Graz, Graz, Austria.* Sponsor: D. Fort.

Halogenated acetonitriles (XANs) were tested as part of a larger mixture toxicity study. Agents tested included the mono (iodo, bromo, chloro), di (dibromo, dichloro) and tri (trichloro) forms. Toxicity was assessed with Microtox at 15, 30 and 45 minutes of exposure with seven concentrations and a control (each duplicated) for each single agent (A, B) and mixture (A:B). Concentration-response data were fit to sigmoid curves using the four-parameter logistic function (4PL) and the five-parameter logistic minus one-parameter (5PL-1P) function. For the 4PL, parameters included minimum effect (0.01 < min < 1.0), maximum effect (max < 100), EC50 and slope, while for 5PL-1P the minimum effect parameter was removed and an asymmetry parameter (s) was added (0.1 < s < 10). The 5PL-1P had a maximum effect constraint (max < 100). Fifteen combinations of mono:di, mono:tri, di:di, or di:tri XANs were tested. Correlation coefficient (r2) values for 5PL-1P curves (mean = 0.9979) were higher than (68%) or equal to (21%) those for the 4PL (mean = 0.9962) for 120 of 135 curves. Improved r2 values were also observed at each exposure duration (e.g., 5PL-1P: mean = 0.9978, 4PL: mean = 0.9961, n = 45). With inclusion of the asymmetry parameter, calculated EC50 values were usually different between functions. This affected calculation of theoretical dose-addition and independence curves and held the potential to alter the conclusion on combined effect. Examination of single agent curves suggested greater asymmetry was related to greater differences in r2 values between the 5PL-1P and 4PL functions, so the 5PL-1P was preferred.

PS 506 PROPENTOFYLLINE ATTENUATES METHAMPHETAMINE–INDUCED DOPAMINE RELEASE IN THE RAT CAUDATE-PUTAMEN.

Z. Binienda[1], B. Gough[1], F. C. Pereira[2] and S. F. Ali[1, 2]. [1]*Neurotoxicology, U.S. FDA/NCTR, Little Rock, AR and* [2]*Medicine, University of Coimbra, Coimbra, Portugal.*

The abuse of stimulant such as methamphetamine (METH) for functional enhancement often leads to dependency. While there are currently no medications approved for treating METH abuse, its pharmacology as an indirect sympathomimetic agent provides opportunities for potential pharmacological strategies for regulation of METH-induced dopaminergic transmission. Propentofylline (PPF), an atypical methylxanthine, is known to modulate glial activity under pathological conditions. It was recently shown that PPF suppressed METH-induced rewarding effects in mice and diminished METH-evoked astrocytic activation in vitro. Here, we have investigated the impact of intra-striatally perfused PPF (20 mM) on the effect of METH (5 mg/kg, i.p.) effect on striatal dopamine (DA) release using brain microdialysis in adult Sprague-Dawley rats. METH was injected after PPF was perfused for 50 min. The extracellular and total concentration of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) were determined using high performance liquid chromatography with electrochemical detection (HPLC-ED). PPF alone induced a three-fold increase in DA extracellular levels 20 min following the beginning of perfusion and attenuated the METH-induced DA peak-effect 50 min after METH injection (from 14- to 5-fold increase). Moreover, PPF dampened the extracellular decrease in DOPAC induced by METH. Extracellular levels of HVA remained unchanged in all experimental conditions. These data indicate that METH-evoked DA release can be attenuated by treatment with PPF.

PS 507 A DOSE VOLUME TOLERABILITY STUDY TO EVALUATE WHETHER CD-1 MICE ARE EXTRAORDINARILY SENSITIVE TO LIPID-BASED DRUG DELIVERY VEHICLES: CAPMUL PG-12, CAPMUL MCM, AND CREMOPHOR EL.

R. Yeager[1], B. Padden[2], J. Lipari[2], J. Maher[3], K. Desino[4], P. Katavolos[5], D. Weddle[5] and K. Sachdeva[1]. *[1]Toxicology, Abbott, Abbott Park, IL, [2]Pharmaceutics, Abbott, Abbott Park, IL, [3]Investigative Toxicology and Pathology, Abbott, Abbott Park, IL, [4]Drug Metabolism, Abbott, Abbott Park, IL and [5]Pathology, Abbott, Abbott Park, IL.*

Lipid-based vehicles are often used to formulate test article for preclinical toxicology studies in order to increase absorption and thereby maximize systemic drug exposures. It remains unclear whether certain lipid-based vehicles alone, in the absence of test article, can produce toxicologically relevant changes *in vivo*. There is anecdotal evidence suggesting that mice are extraordinarily sensitive to certain lipid-based vehicles, including Capmul PG-12, which may compromise the interpretation of potential test article-related changes in mice. In the present study, nine week old male CD-1 mice were orally administered 0.9% saline, corn oil, Capmul PG-12:corn oil (80:20, w/w), Capmul MCM:corn oil (80:20 w/w), or Cremophor EL:saline (10:90, w/w) once daily for 14 consecutive days at dose volumes of 2, 5, 10, or 15 mL/kg. The saline and corn oil were non-lipid and lipid controls, respectively, and the three lipid-based vehicles were prepared in proportions commonly used in formulations for preclinical toxicology studies. The following parameters were evaluated: survival, clinical signs, body weight, food consumption, hematology, clinical chemistry, and histopathology. *In vitro* studies were also conducted to evaluate whether these vehicles can 1) bind and activate receptors, 2) inhibit activity of major CYP isozymes, or 3) interact with the drug efflux transporter P-gp. The present study evaluates whether Capmul PG-12, Capmul MCM, or Cremophor EL produce toxicologically relevant changes when administered to CD-1 mice for 14 consecutive days at various dose volumes.

PS 508 ESTABLISHMENT OF 2-STEP ULTRA-SHORT-TERM CARCINOGENICITY TEST IN CB6F1 TG RASH2 MICE - STUDY ON SEVERAL PROMOTERS-.

K. Urano[1], M. Kawabe[2], M. Suguro[2], T. Numano[2], M. Yasuda[1], H. Tsutsumi[1] and F. Furukawa[2]. *[1]Central Institute for Experimental Animals, Kawasaki, Japan and [2]DIMS Institute of Medical Science, Inc., Ichinomiya, Japan.*

Short-term carcinogenicity studies using rasH2 mice have recently been performed in place of the conventional 2-year carcinogenicity studies. At SOT 2010, we reported that the latent period of skin tumor induction in rasH2 mice after DMBA + TPA treatment was 4 weeks. In this study, several test chemicals were applied to rasH2 mice after DMBA application in order to clarify the carcinogenicity potential. 4-Vinyl-cyclohexane diepoxide (4VCD), known as a skin carcinogen at 0.5 or 2.5 mg x 5 times/week, and diisopropylcarbodiimide (DC), benzethonium chloride (BC) or oleic acid diethanolamine condensate (OADC), known to have no promoting effects, at 40 mg/kg x 5 times/week, 1.5mg/kg x 5 times/week or 30 mg/kg x 7 times/week, respectively, were applied to shaved dorsal skin of female rasH2 mice from one week after 50μg DMBA application. Vehicles (99.5% ethanol for OADC or anhydrous ethanol for DC and BC) were also applied after DMBA application. Test chemicals treated groups without DMBA were also established. All mice were sacrificed at 12 weeks after DMBA application and histopathological examination was carried out for dorsal skin. All DMBA treated groups except for that treated with 99.5% ethanol had induced skin tumors at 4 weeks after DMBA application. Incidences/multiplicities of skin tumors including squamous cell hyperplasia, squamous cell papilloma or squamous cell carcinoma were 20~30%/0.2~0.4 in these groups. No tumors were observed in the groups without DMBA application. Further studies appear to be necessary since rare tumors were observed in 4VCD treated groups, or unexpected tumors were observed in DC, BC, OADC and vehicle treated groups.

PS 509 THE EFFECT OF CLOZAPINE TREATMENT ON NEUTROPHIL KINETICS IN SPRAGUE-DAWLEY RATS.

A. Lobach and J. Uetrecht. *Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada.*

Background: Clozapine is a very effective antipsychotic agent but its use is limited by the risk of drug-induced agranulocytosis. Although we have been unable to reproduce clozapine-induced agranulocytosis in animals, clozapine treatment was found to result in a marked increase in the rate of neutrophil turnover in rabbits. However, the use of rabbits limits the availability of reagents and mutant strains and so we sought to extend these studies to rats and mice.
Methods: Female Sprague-Dawley rats were treated with clozapine (30 mg/kg/day) or saline, i.p. for up to 10 days and blood was collected at various time points. Serum levels of granulocyte-colony stimulating factor (G-CSF) were measured by ELISA. White blood cell and differential counts were obtained manually from whole blood samples. For kinetic studies, following the 4th clozapine treatment rats were administered a single dose of bromodeoxyuridine (BrdU, 100 mg/kg). The time at which BrdU was administered was designated as time 0. Leukocytes were isolated from whole blood and flow cytometry was used to detect BrdU-labeled neutrophils.
Results: As early as Day 2, rats treated with clozapine displayed elevated neutrophil counts and decreased lymphocyte counts in comparison to control. Serum levels of G-CSF were also elevated in the treated group as early as Day 2. The number of BrdU-labeled neutrophils was increased in the peripheral circulation of treated rats, indicating that clozapine influenced the rate of release of neutrophils from the bone marrow.
Conclusions: This data indicates that neutrophils in clozapine-treated rats had a shorter transit time through the bone marrow. The mechanism is unclear, however other studies suggest that this is the result of clozapine-induced apoptosis. Clozapine-induced agranulocytosis may occur in cases where this damage leads to an immune response. This research was supported by grants from CIHR.

PS 510 TROGLITAZONE-INDUCED LIVER INJURY IN CHIMERIC MICE WITH HUMANIZED LIVER.

M. Kakuni[1], M. Morita[2], Y. Katoh[1], M. Nakajima[2], C. Tateno[1] and T. Yokoi[2]. *[1]Study Service Department, PhoenixBio Co., Ltd., Higashi-Hiroshima, Japan and [2]Division of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan.*

Troglitazone is a thiazolidinedione antidiabetic drug that was withdrawn from the market due to its association with idiosyncratic severe liver injury. Troglitazone has never induced liver dysfunction in numerous in vivo tests using general experimental animals. We previously established a chimeric mouse with humanized liver whose replacement index with transplanted human hepatocytes is up to 95%. The aim of this study was to establish an animal model of troglitazone-induced liver injury and to elucidate the underlying mechanism of the hepatotoxic effect of troglitazone. Troglitazone was daily administered to the chimeric mice for 14 and 23 days (1,000 mg/kg/day, po). We found that troglitazone caused significant ALT increases in both 14 or 23 day groups by 1.6- and 3.2-fold compared with the control group. Co-administration of L-buthionine sulfoximine (10 mM in drinking water), an inhibitor of glutathione synthesis, prevented the increase of ALT observed in the troglitazone-treated groups. To elucidate the mechanism of the onset of the liver injury, hepatic GSH, oxidative stress markers and phase I and phase II drug- metabolizing enzymes were analyzed. However, none of those analyses suggested the mechanism of the troglitazone-induced liver injury. In conclusion, we successfully established a mouse model of troglitazone-induced liver injury by using chimeric mice with humanized liver, which provide a new animal model for the study of idiosyncratic drug-induced liver injury.

PS 511 CHARACTERIZATION OF IRON OVERLOAD IN RATS AS A POTENTIAL MODEL OF HUMAN HEMOCHROMATOSIS.

S. J. Wildt[1], T. Bartnikas[2], M. D. Fleming[2] and D. M. Cooper[1]. *[1]Laboratory Animal Medicine, Harlan Laboratories, Indianapolis, IN and [2]Department of Pathology, Children's Hospital Boston, Boston, MA.* Sponsor: A. Jackson.

Human hemochromatosis (HHC) is a genetic disorder characterized by progressive iron overload in parenchymal cells in many organs, particularly in the liver. Sporadic iron overload has been reported in rats, but the mechanism is not understood. Routine phenotypic analysis on a population of Wistar rats revealed a low incidence of iron overload (<1%). To characterize the mechanism of this observation, samples of serum, spleen, liver, and tail tissue were collected from 74 male and 58 female rats for iron overload screening and gene expression analysis. This subpopulation was designated Hsd:HHCL. Of the 132 animals screened, two male rats exhibited a high serum iron level, transferrin saturation and liver iron level but normal spleen iron level. Liver iron accumulation occurred predominantly in the cytoplasm of hepatocytes in a periportal distribution in one affected male rat but displayed a more diffuse distribution in the other. RNA levels of hepcidin, a hepatic peptide that inhibits dietary iron absorption and macrophage iron recycling, and protein levels of transferrin receptor 2, a factor linked to HHC, were inappropriately low given the level of iron overload in these affected rats. Our results indicate

that the iron overload observed is likely due to a genetic component. Although the incidence of iron overload in the Hsd:HHCL rat population was very low, this could be due to a rare allele, possibly with incomplete penetrance. These findings are unique in that there are no known models of rat hemochromatosis other than ones that are diet-induced and all models of HHC are mouse knock-out models. As mice are not always suitable models for human disease research, the Hsd:HHCL rat could be a better model of human hemochromatosis. This model is potentially useful for development of treatments for HHC and for understanding the molecular mechanisms for regulation of iron metabolism.

PS 512 PAIN ASSESSMENT IN MONOSODIUM IODOACETATE (MIA)-INDUCED OSTEOARTHRITIS (OA) MODEL.

R. Samadfam, L. Chouinard and S. Y. Smith. *Toxicology, Charles River Preclinical Services, Montreal, QC, Canada.* Sponsor: M. Vézina.

Major findings in basic science of pain have failed to bring new drugs into clinical practice, partly due to the fact that the preclinical models used for pain assessments do not adequately represent the clinical condition. A potentially useful approach to evaluate antinociceptives is using animal models with diseases similar to humans such as OA. The objectives of this study were to determine the optimal dose of MIA to induce joint discomfort in rats and develop/validate relevant pain endpoints. Measurement of dynamic weight bearing (DWB) quantifies a spontaneously emitted behavior, providing more clinically relevant results compared to reflexive measurements (Von Frey filament (VFF) or pinch). Adult rats were assigned to three groups, each receiving an intra-articular injection of saline or MIA at 1 or 3 mg/dose in the right knee. Pain was evaluated at Day 14 using: body position, gait, toe pinch, modified grip strength test VFF and DWB. Animals with more severe symptoms of OA were treated with Naproxen and pain was evaluated once predose, 4 and 6 hours post dose. Animals treated with 1 mg/dose generally had less severe OA symptoms with normal body position and gait and only trends of an altered weight bearing of the right rear paw compared to controls. Animals treated with 3 mg/dose had more severe OA had normal gait and clearly showed signs of joint discomfort (right rear paw) as measured by DWB. Modified grip strength test indicated the right rear paw was weaker than the untreated left rear paw. The VFF test was the most sensitive endpoint for this model with significant differences between the right and left rear paws. Naproxen reduced pain in the majority of the 3 mg/dose animals as evidenced by balanced weight distribution and comparable use of both rear paws. These results indicate that reflexive pain measures were successfully measured at 1 mg/dose. However, the 3 mg/dose produced consistent quantitative changes and was considered the optimal dose of MIA to assess pain in a more clinically relevant condition (spontaneously emitted behavior) using DWB.

PS 513 PROTEOMIC ANALYSIS OF MAINSTREAM CIGARETTE SMOKE-EXPOSED FISHER RAT NOSES IN A SHORT-TERM STUDY.

C. A. Carter and M. Misra. *Life Sciences, Lorillard Tobacco Company, Greensboro, NC.*

A short-term 5 day nose-only smoke exposure study was conducted in Fisher 344 rats to identify smoke-induced nose protein changes. Groups of 10 male and female 5 wk old rats were assigned to 1 of 4 exposure groups. Animals received filtered air, or 75, 200 or 400 total particulate matter (TPM) mg/m^3 of diluted 3R4F Kentucky reference cigarette mainstream smoke. Exposures were conducted for 3 hrs/day, for 5 consecutive days. Half of the harvested nose tissue was decalcified and processed for pathology, while the soft tissues were removed from the other half and processed for proteomic analysis. We hypothesized that inflammation pathways are activated in nasal tissues. Changes in smoke-treated animals included loss of ciliated epithelium, hyperplasia and metaplasia of the respiratory epithelial septum, and increases in serous exudate and inflammation. Nose lysates from control vs. treated animals were screened for 800 proteins using antibody-based microarray technology and the 18 most changed proteins were evaluated by Western blot. Proteins that were expressed at high levels in the nose and are markedly increased or decreased by smoke treatment depended on dose and gender and included: heat shock protein 90 (Hsp90), serine/threonine protein kinase 33 (STK33), protein kinase C-delta and zeta, p53 apoptosis effector related to PMP-22 (PERP), and interleukin-1 receptor-associated kinase-like 2 (IRAK2). Thus, smoke affected protein pathways that are involved in stress, inflammation, proliferation, apoptosis, and transformation. Inflammatory mediators were the common change observed both with proteomics and pathology. Proteomics is a powerful method for identifying key pathways affected by smoke exposure. Changes in identified proteins affected by smoke exposure may induce functional changes in the nose and could serve as early indicators of nasal damage and associated disease.

PS 514 FIRST IN MONKEY (FIM) PERFORMED TRACKING OF MAGNETIC CAPSULE INSIDE THE GASTROINTESTINAL TRACT WITH 3D-MAGMA.

H. Richert[2], S. H. Korte[1] and S. Abert[2]. [1]*Covance Laboratories GmbH, Muenster, Germany and* [2]*Matesy GmbH, Jena, Germany.* Sponsor: G. Weinbauer.

In order to reach a maximum benefit in oral drug absorption new substances shall be tested for their permeability in different regions of the human GI-tract. Furthermore, for toxicological investigations of oral drug forms it is often necessary to release a new candidate within a specific region inside the gastrointestinal tract. Remote controlled drug release into the gastrointestinal tract can be done with the innovative system 3D-MAGMA/MAARS, which acts magnetically for capsule localization and for drug release.
In this first study one fasted, non sedated male Cynomolgus monkey (macaca fascicularis) was used to test and visualize the gastrointestinal transit of a magnetic capsule. With the magnetic monitoring system 3D-MAGMA the capsule was monitored over 100 min every 20 min for 2 to 6 min. The system measured the 3D-position of a magnetic marked capsule and estimated in real time the travelled path and local motility patterns of the GI tract. Following results could be achieved: Within the first 90 minutes after capsule application a dominant frequency of approx. 2.7 to 3.3 min-1 could be determined and the three-dimensional capsule position was detected in the region of the stomach. According to the literature, the slow waves in the stomach have a frequency of about 3 min-1 in Cynomolgus monkeys (similar to humans), so the capsule was surely inside the stomach. 90 minutes after start of the investigation the dominant frequencies and the capsule position changed. The known intestinal slow waves with a frequency of about 12 min-1 could be found, so that we could assume that the capsule had successfully traveled into the small intestine. The animals breathing frequency of about 50 min-1 was always visible.
CONCLUSION: It seems to be feasible to identify the capsule position during the intestinal transit by evaluating the occurring motility pattern combined with the 3d position of magnetic capsule. Complete visualizations of intestinal passages in non-sedated monkeys are difficult because of short measurement intervals.

PS 515 TOXIC EFFECTS OF ETHYL TERTIARY BUTYL ETHER EXPOSURE IN CYP2E1 KNOCKOUT MICE.

R. Wang[1], Y. Yanagiba[1], K. Ohtani[1], M. Suda[1], Z. Weng[1] and T. Nakajima[2]. [1]*Japan National Institute of Occupational Safety and Health, Kawasaki, Japan and* [2]*Nagoya University School of Medicine, Nagoya, Japan.* Sponsor: N. Mei.

Ethyl Tertiary Butyl Ether (ETBE) is used as a fuel oxygenate in gasoline. Previous studies with animals indicated that the toxicity of this chemical is low. ETBE is metabolized in body by CYP2E1 as well as CYP2A6 to two primary intermetabolites, acetaldehyde and t-butyl alcohol, which are believed to be toxic. Therefore, the transformation of ETBE in body is critical for its toxicity. In this study, we used CYP2E1 knockout mice to investigate if there is any change in the susceptibility to the toxic effects of ETBE. METHODS: Wild type SV129 mice and its CYP2E1 knockout mice were exposed to ETBE at 0, 500, 1750 and 5000 ppm ETBE, 6 hr/day and 5 days/week, for 6 weeks. Blood, liver and epididymides were sampled 20 hr after the last exposure. RESULTS: Increase in the ratio of liver/body weight was only observed in 5000 ppm exposure of both genotypes of mice; as for the effects on hemotology, there was a tendency of dose-dependent increase in the erythrocyte counts and hemoglobin concentrations in the groups of exposure of either wild type or knockout mice, but statistical differences were only found between the middle and high doses groups and the controls of wild type mice. ETBE exposure also affected the motility of sperm, as showed by the significantly low percentages of motile and progressive sperm with the two high doses of ETBE. The reproductive effect was observed in both types of mice at approximately similar extent. These results indicated that while CYP2E1 may play a role in the metabolism of ETBE, lack of the enzyme has little influence on the toxic effects of the chemical. We thank Ms. S. Watanabe for her assistance in the manipulation of animals.

PS 516 THE INDIVIDUAL AND INTERACTIVE EFFECTS OF LIPOPOLYSACCHARIDE AND DEOXYNIVALENOL ON FREE FATTY ACIDS IN THE LIVER AND BRAIN OF RATS.

I. Ross[1], T. Boyle[1], W. D. Johnson[1], L. H. Garthoff[1], S. M. Ahn[1], M. W. O'Donnell[2] and C. S. Kim[1]. [1]*U.S. FDA, Laurel, MD and* [2]*Biostatistics Branch, Laurel, MD.* Sponsor: H. Luu.

Post-translocation modification by the palmitate is crucial for the correct targeting and function of many proteins in cells. Mutation of palmitation site makes palmitation resistant and increases neuronal toxicity in the brain. Palmitoylation of immune effectors is known to be crucial for host resistance to infections. This research

involved the use of an acute inflammatory co-exposure model to characterize the biochemical and physiological interactions between lipopolysaccharide (LPS) and deoxynivalenol (DON) when administered at a low dose that alone would not cause overt toxicity. Male rats were divided into 4 groups. Group 1 was administered an IP dose of 10 mg DON/kg; group 2: 83 μg LPS/kg; group 3:10 mg DON and 83 μg LPS/kg; group 4: 1 mL saline/kg. Animals were sacrificed at 3, 24, and 72 hours after dosing. The liver and brain were harvested and the free fatty acids extracted and analyzed by gas chromatography. There was no significant change in the brain. In the liver of group 1, palmitic acid decreased by 21.56% between 3 and 24 hr and increased 24.42% between 24 and 72 hr. In group 2, elaidic acid decreased 54.42% between 3 and 24 hr, and 6.88% between 24 hr and 72 hr. Stearic acid decreased 33.33% between 3 and 24 hr and 60.32% between 24 and 72 hr. There was no significant change in group 3 and 4. This fatty acid profile of a decrease and increase in palmitic acid with DON, and the decrease of elaidic and stearic acids with LPS are indications of the participation in the palmitoylation process by the early exposure of the cells to the toxins. Absence of active changes in the levels of affected free fatty acids during co-exposure of LPS/DON over time suggests a disappearance of defense mechanism against organ failure and innate immunity which warrants further investigations on the crucial role of palmitoylation in infection and chemical toxicity.

PS 517 FEASIBILITY OF APPLYING DRIED BLOOD SPOT (DBS) SAMPLING IN EXPLORATORY TOXICITY STUDIES IN THE RAT.

W. Scott[1], R. Hunter[2], Z. Shen[2], J. May[1], L. Liu[1], S. Vekich[2], W. Huang[1], R. Rahavendran[2] and A. Sacaan[1]. [1]*Drug Safety Research and Development, Pfizer Inc., San Diego, CA and* [2]*Pharmacokinetics Dynamics and Metabolism, Pfizer Inc., San Diego, CA.*

Rodent toxicity studies employ repeat blood sampling for toxicokinetic (TK) assessment to accurately determine drug concentration and disposition. Typically this is done using satellite groups to avoid the risk of compromising main study animals. However, Dried Blood Spot (DBS) technology may enable the use of the main toxicity animals for TK sampling. This is beneficial by reducing the number of animals used on studies as well as enabling direct comparison of TK profile with clinical findings in the same animals. In this study we evaluated the feasibility of DBS technology using the main toxicology study animals.
Animals were divided into two groups and were administered vehicle once daily via the oral route for 28 days; one group served as control and the other underwent serial blood sampling up to 1.1 mL (total) over three sampling occasions (Days 1, 14 and 28). Clinical observation, histopathology and clinical chemistry were evaluated. Data demonstrated that sampling up to 1.1 mL of blood did not lead to any remarkable findings. We then conducted a second series of studies comparing TK profiles of several compounds using plasma and whole blood - DBS. The majority of the compounds tested had very comparable TK profiles further validating the use of DBS. Lastly we conducted a study comparing the route of drug administration and/or blood collection on the TK profile. In this study, Imatinib was administered orally or intravenously to rats and blood was simultaneously sampled from three different collection routes (jugular vein via an indwelling catheter, lateral tail vein, and saphenous vein). Results revealed that there were no statistical differences in the calculated TK parameters regardless of the route of blood collection. These data are encouraging and support the use of the main study toxicology rats for TK sampling thus eliminating the need for satellite TK groups in accordance with the 3R's initiative.

PS 518 APPLICATION OF PREDICTION INTERVAL BASED MIXED-EFFECT MODELS TO EVALUATE MONKEY BODY WEIGHT CHANGE IN PRECLINICAL TOXICOLOGY STUDIES.

D. Zhao[1], R. Yeager[1], Y. Lan[2], C. Lin[2] and M. Duvall[1]. [1]*Toxicology, Abbott, Abbott Park, IL and* [2]*Statistics, Abbott, Abbott Park, IL.*

Cynomolgus monkeys are an important and widely used species in preclinical toxicology studies in drug research and development. Body weight effect and its correlation to toxicity can be difficult to evaluate due to small sample size and/or large inter- and intra-animal variability. Previously, pooled body weight data from more than 550 individual control monkeys from 56 preclinical toxicology studies of 1-, 3- or 9-month duration were used to establish tolerance intervals in a mixed-effect model. In the present study, the mixed-effect model was modified to incorporate prediction intervals derived from the same control data set. These intervals provide the normal bounds for body weight change from baseline in a control monkey over time at a stated confidence level (dependent on study duration). In the present study, body weight data, clinical observations, and associated pathology from more than 500 compound-treated monkeys from 25 studies were analyzed. Although the model is shown to be of limited utility when applied to shorter term studies (≤1-month duration), the robust data set and prediction intervals are a reliable aide in interpreting the toxicological relevance of body weight effects in 3-month and 9-month monkey toxicity studies. Moreover, the results suggest that the model has utility in quantitatively predicting the trend of individual body weight change in order to make scientifically sound decisions during study conduct.

PS 519 LONGITUDINAL COMPARISON OF SPONTANEOUS OCULAR LESIONS IN THE NORMAL RAT AND MOUSE ON CHRONIC TOXICOLOGY STUDIES.

K. Tenneson, N. Cicciu and M. Vézina. *Toxicology, Charles River Preclinical Services, Montreal, QC, Canada.*

Ophthalmologic examinations are commonly conducted prior to the initiation of dosing in rodent toxicology studies to confirm ocular health and are repeated throughout the course of the study. We conducted a review of control animal data for the Sprague-Dawley and Wistar Han rat, and the CD1 mouse from chronic toxicology studies to determine the background incidence and progression of changes with age. Corneal opacities were the most common observation in both rat strains and mice ≤20 weeks of age and did not increase in incidence with age. There was a low incidence of lens opacities in animals ≤20 weeks of age (10% in Sprague-Dawley rats and 0% in CD1 mice). Lens opacities demonstrated a progressive increase in incidence in both strains of rats and mice affecting up to 25% of rats and 75% of mice ≤ 2 years of age. Focal chorioretinal atrophy was observed in both strains of rats and mice at a low incidence (approximate rate of ~1.5%) in prestudy examinations and also developed during the course of treatment period appearing as late as 1 year of age. In conclusion, we characterized findings on ophthalmologic examinations over the life span of two strains of rats and mice on chronic toxicology studies to facilitate the recognition of test article-related changes.

PS 520 COMPARISON OF ANESTHETIC EFFECTS ON ELECTRORETINOGRAMS (ERG) IN DOGS.

G. Glazier, D. Martel, T. Halle, S. Duval, T. Bryant and M. Vézina. *Toxicology, Charles River Preclinical Services, Montreal, QC, Canada.*

The objective of this study was to investigate a suitable alternative for ketamine/xylazine anesthesia, known for its minimal effects on the ERG, but also for prolonged recovery times and potential drug interactions. The effect of 2-3% isoflurane (IM) or 0.8 mg/kg/min propofol (PM), both in combination with 40 μg/kg medetomidine on the ERG response in dogs was compared to that of 35.0 mg/kg ketamine/2.0 mg/kg xylazine (KX). Four dogs were anesthetized on up to 4 occasions (minimum 4 days washout period) with each anesthetic combination. Each ERG occasion consisted of a series of scotopic single flash stimuli following a dark adaptation period of at least 1 hour (a. average of 5 single flashes at -30 dB, 10 seconds between flashes; and b. -10 dB, 15 seconds between flashes; c. average of 2 single flashes at 0 dB (2.0 cd-s/m2), 120 seconds between flashes). Amplitudes with IM, were decreased (A and B waves) and delayed (A wave only for -10 and 0 db) compared to those obtained with KX. In contrast, PM amplitudes were generally increased (with the exception of the A wave for the 0 db test) with a faster response time for the B-wave for all tests. PM had a milder inhibition on the A-wave at 0 db with a 10% reduction in amplitude and 10% increased implicit time compared to the IM at 42% reduction of amplitude and 34% increased implicit time. Ranges for amplitude and/or implicit time with IM were generally more variable than those with KX and PM, whereas PM ranges were more similar to those obtained with the KX. PM produced less variable data than IM as well. In conclusion, PM produced the most similar data to those obtained with KM, but with an increased sensitivity to the light stimulus, and was considered a suitable alternative anesthetic agent for ERG testing when KX anesthesia is contraindicated. In addition, IM anesthesia is not recommended for ERG data collection.

PS 521 APPLICATION OF THE *ras*H2 MOUSE IN CARCINOGENESIS AND BIOMARKER STUDIES.

Y. Shimamura[1], R. Arai[1], A. Sunohara[1], F. Baba[1], K. Urano[2], H. Tsutsumi[2], M. Ito[2], A. Hirayama[1], M. Sugimoto[1], T. Soga[1], T. Nomura[2] and M. Tomita[1]. [1]*Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata, Japan and* [2]*Central Institute for Experimental Animals, Kawasaki, Kanagawa, Japan.*

The *ras*H2 mouse is a hemizygous transgenic animal used in 26-week carcinogenicity studies for regulatory purposes. We investigated the metabolite profile of this mouse model as a key factor in carcinogenesis using high throughput capillary electrophoresis/time-of-flight mass spectrometry (CE-TOFMS). N-methyl-N-nitrosourea (MNU, 37.5 mg/kg) was administered intraperitoneally to *ras*H2 mouse

(n=50) and non-Tg mouse (n=20) at 7 weeks of age. Citrate buffer at pH 4.5 (10ml/kg) was also injected intraperitoneally in *ras*H2 (n=10) and non-Tg (n=10) mouse as a negative control agent. Serum samples were obtained from each mouse at 2-week intervals, and metabolites were measured by CE-TOFMS. When all data were classified according to pathological findings, some peaks that suggested possible carcinogenic substances were found in the MNU dosed *ras*H2 mouse. At least one signal was detected as a possible biomarker. Further investigations will be required, but the CE-TOFMS could be a powerful tool for screening carcinogenic biomarkers together with the *ras*H2 model.

PS 522 DEVELOPMENT OF INDUCIBLE *IN VIVO* RNAi MOUSE MODELS FOR TARGET VALIDATION.

J. Hitchcock[1], V. Beuger[2], H. Kissel[2], N. Pullen[1] and A. Rossi[1]. [1]*Pfizer Ltd., Sandwich, Kent, United Kingdom and* [2]*TaconicArtemis GmbH, Cologne, Germany.* Sponsor: M. Sharpe.

Inducible RNAi systems allow the rapid expression of functional small hairpin (sh) RNA molecules with tight on/off regulation for the purpose of silencing target genes. The key advantages of an inducible *in-vivo* RNAi system include a) the ability to study the loss of function of essential genes which would otherwise result in knockout lethality b) less concerns around developmental affects compared with traditional knockout models. A strategic alliance was formed with TaconicArtemis to help evaluate the utility of this technology for target validation. The aim of the project was to develop inducible RNAi mouse models against a specific target gene, to aid in evaluating and characterising the technology, and to help understand the safety of the target. The target selected was Cyr61/CCN1, a member of the CCN family of secreted matricellular proteins, which has a role in proliferation, angiogenesis, inflammation and fibrosis. Studies of Cyr61 have been hampered by the lack of relevant animal models, as the murine knockout has an embryonic lethal phenotype. The first step in generation of the inducible mouse models was the selection of potent shRNA's designed against the Cyr61 gene. This proved to be quite challenging and a number of strategies had to be employed, including the use of different shRNA selection algorithms, and designing against the untranslated region (UTR) of the gene. This has been shown elsewhere as a useful approach for gene knockdown studies (McManus et al, 2002). Three shRNA's exhibiting potent knockdown in mouse embryonic stem cells were selected. shRNA-1, shRNA-20 and shRNA-21 caused 73%, 75% and 79% knockdown at the gene level, measured using qRT-PCR. Furthermore the shRNA's caused 56%, 71% and 77% knockdown of Cyr61 protein, as shown by Western blotting. Animal generation using these potent shRNA's has since been undertaken and resulting phenotypes are currently being analysed. Mcmanus MT. et al., 2002. Gene silencing using microRNA designed hairpins. RNA 8:842-850

PS 523 METABOLOMIC ASSESSMENT OF TEMPORAL CHANGES INDUCED DURING THE COURSE OF A TYPICAL OVERNIGHT FAST IN THE SD RAT.

D. G. Robertson, S. Stryker and J. Vassallo. *Discovery Toxicology, Bristol-Myers Squibb Company, Princeton, NJ.*

Fasting induces marked changes in the serum metabolomic profile of SD rats. This study examined the temporal metabolic changes induced by fasting of up to 16 hours duration. Groups of 5 male rats were fasted for 2, 4, 8, 12 or 16 hours duration. A group allowed ad libitum access to food served as a control. All groups were sacrificed at the end of the dark cycle (DC-6AM). One additional group, allowed ad libitum access to food, was sacrificed at the end of the light cycle (LC-6 PM). Animals were weighed at the onset and termination of their fasting periods with urine collected during the duration of the fast and serum collected at termination of the fast for metabolic analysis. DC rats gained approximately 5% BW while 16 hr fasting animals lost approximately 8%BW. From 8-16 hrs of fasting, rats lost approximately 1 g/hr with urine production of ~ 0.9 ml/hr as compared to urine production of ~0.3 mL/hr in DC controls. Serum GLU and TRIG dropped sharply after 2 hours (by 38 and 64%, respectively) with little subsequent change. TRIG levels in LC controls were decreased relative to DC controls (by 67%) while GLU levels were similar. Pronounced metabolic effects were induced by fasting though in many case LC did not vary from DC even though food consumption was markedly decreased during LC. Transcriptional evaluation of fast-twitch muscles indicated time-dependent induction of muscle specific ubiquitin ligase atrogin-1 with more than a 5-fold increases at 16 hours compared to DC. Consistent with these changes, serum 3-methylhistidine, a marker for myofibrillar protein degradation, showed a time dependent increase (up to 3X) after 8 hours of fasting. With some notable exceptions, metabolic profiles of LC were more similar to DC than to fasting animals (at any time point) suggesting complex metabolic interplay between diurnal metabolic responses and those metabolic changes induced by fasting.

PS 524 BACKGROUND OCULAR CHANGES FOLLOWING TRANSVITREAL SUBRETINAL DOSING IN THE RHESUS AND CYNOMOLGUS MONKEY.

A. Préfontaine, M. Bussières and M. Vézina. *Toxicology, Charles River Preclinical Services, Montreal, QC, Canada.*

Subretinal dosing allows for localized, controlled exposure within the eye and is typically used for gene or cell-based therapies. The transvitreal approach allows precise placement of the injection. The background changes for rhesus and cynomolgus monkeys following a 100 µL subretinal injection of saline are described. Animals were anesthetized and under an operating microscope a self-sealing 25G port system allowed both a light source and 41G injection needle into the eye to create a bleb (injection) adjacent to the macula. Ophthalmology was evaluated up to 8 times and H&E stained sections were evaluated at 13 wks. There was occasional slight reflux from the bleb into the vitreous and retinal/choroidal hemorrhage subsequent to the procedure. Areas of hemorrhage progressively resolved by Day 5 and the blebs receded by Day 8. Traumatic, procedure-related signs of inflammation; anterior chamber cell/flare and anterior vitreous chamber cell-like opacities resolved within 13 wks. There was slight retina/choroid pigment variation associated with the bleb. Microscopic evaluation of the bleb area indicated very slight retinal atrophy of the photoreceptors/outer nuclear layer, fibrosis of the choroid, and retinal folds/rosettes. Needle tract lesion and lens degeneration were occasionally noted. These changes were considered related to the experimental procedure (including transient retinal elevation caused by the bleb). There were no microscopic findings adjacent to the bleb. In conclusion, transvitreal subretinal injection of 100 µL produced similar and consistent mild findings in both rhesus and cynomolgus monkeys that were transient or stable over the course of 13 wks. Characterization of the procedure-related findings facilitates the recognition of test article-related changes.

PS 525 DOSE RESPONSE OF DIURON-INDUCED URINARY BLADDER HYPERPLASIA IN MALE WISTAR RATS.

A. F. Cardoso, S. M. Ihlaseh, M. S. da Rocha, M. G. Nascimento, J. V. de Camargo and M. C. de Oliveira. *Pathology, Sao Paulo State University - UNESP - Medical School, Botucatu, Brazil.*

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a urea-derived herbicide, increased the incidences of urothelial tumors in both genders of Wistar rats fed 2500 ppm in a two-year bioassay. Accordingly, USEPA has categorized diuron as a "known/likely" human carcinogen. The accepted non-genotoxic mode of action (MOA) of diuron encompasses urothelial necrosis induced by direct cell exfoliation or cytotoxicity caused by diuron and/or its metabolites, followed by regenerative cell proliferation and sustained urothelial hyperplasia that may favor neoplasia development. This study, approved by the local Committee for Ethics in Animal Experimentation, was designed to evaluate the dose-response of diuron regarding urothelial lesions as detected by histology, immunohistochemistry and scanning electron microscopy (SEM). Six groups of male Wistar rats were fed diuron for 20 weeks mixed in the diet at 0, 60, 125, 500, 1250, or 2500 ppm. One hour before euthanized, rats were injected with BrdU (100 mg/kg body weight, i.p.). Histological analysis of the urinary bladders revealed that the two higher doses induced a significantly increased incidence of simple hyperplasia, a preneoplastic urothelial lesion. Urothelial cell proliferation was documented also by BrdU labeling. By SEM, the incidences and severity of lesions were significantly greater in the 500 and 1250 ppm groups (5/10 and 7/10, respectively) than in the lower-dose and control groups (respectively, 1/10, 2/10 and 0/10). Although numerically increased, the incidence of lesions in the 2500 ppm group (4/10) did not differ significantly from the control. The present study documented a dose-response influence of diuron on the rat urothelium, with a no observed effect level (NOEL) of 125 ppm. Support: FAPESP (Proc. 06/60506-1 and 07/55901-1).

PS 526 BIOAVAILABILITY AND DISTRIBUTION OF SOIL BOUND POLYCYCLIC AROMATIC HYDROCARBONS IN SWINE.

R. E. Peters[1], S. D. Siciliano[1, 2] and M. Wickstrom[2]. [1]*Soil Science, University of Saskatchewan, Saskatoon, SK, Canada and* [2]*Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada.* Sponsor: L. Weber.

Polycyclic aromatic hydrocarbons (PAHs) are common soil contaminants introduced to the environment through fuel spills and incomplete combustion. These contaminants are of concern for human exposure, especially in toddlers who are at high risk due to the quantity of soil they ingest as well as their sensitivity to carcinogens. Traditionally, the oral bioavailability of PAHs in soil (defined as PAH ab-

sorption in the gastrointestinal (GI) tract following an oral administration) is assumed to be 1. Following exposure, PAHs are widely distributed through the body. It is known that different routes of exposure (dermal, oral and inhalation) lead to different distribution patterns in the body, but little is known about the effect the exposure vehicle has on the distribution of PAHs. The uptake and tissue distribution pattern of PAHs from eight different contaminated soils was assessed. This study was conducted to measure the oral bioavailability of a suite of PAHs in soil using the juvenile swine model. Swine were dosed with 5g of reference soil containing 7 PAHs of interest in wide ranging concentrations, and sacrificed at various times post-dosing to determine the time course of PAH uptake from the GI tract. PAH concentrations were measured in serum, GI contents and liver samples using HPLC coupled with fluorescence detection. The mean bioavailability of PAHs in soil was calculated to be 0.20 based on area under the curve (AUC) measurements. Tissue distribution between swine dosed with PAHs in an acetonitrile standard solution differed compared to swine dosed with PAHs sorbed to soil. The majority of the PAHs in the swine dosed with the acetonitrile carrier were found in the blood (97%) at two hours, while PAH distribution following dosing with contaminated soil averaged 68% of the PAHs in the serum, 12% in the liver and the remaining 20% in the GI tissue. Further, tissue distribution differed between soil samples. The implications of these different tissue distributions on mammalian risk estimates will be discussed.

PS 527 CENTELLA ASIATICA AN EMERGING ALTERNATIVE TO ALZHEIMER'S DISEASE.

M. Ahuja, M. Dhanasekaran, M. A. Buabeid, V. Suppiramaniam and E. A. AbdelRahman. *Harrison School of Pharmacy, Auburn, AL.*

Introduction: Amyloid-beta (Aβ) toxicity is involved in neurodegeneration leading to the loss of memory in Alzheimer's disease (AD). Various synthetic drugs and alternatives medicines (herbal drugs) are currently tested for their therapeutic efficacy to successfully treat this non-curable disorder. Centella asiatica is one such herb that has shown promising results. However, the mechanism by which it combats Aβ induced neurotoxicity is unknown.
Objectives: To investigate the neuroprotective effects of Centella asiatica extract (CAE) against Aβ induced neurotoxicity using PSAPP double transgenic mice.
Experimental Procedures: CAE mixed in rodent chow, in two doses was administered to PSAPP mice for eight months. Animals were sacrificed and brains were dissected, hippocampus and cortex were removed. Aβ deposition (1-40 and 1-42) as well as enzymatic activity of beta secretase (Elisa Kit) was estimated. Neurotransmitter estimation was performed in the cortex. The alteration in the cellular signaling pathways (ERK MAP kinase, STAT and CREB pathways were analyzed by multiplex microbeads method and with western blot analysis. ANOVA and Dunnett's test were used to compare the results within the different groups.
Results and Conclusion: The significant increase in beta-secretase activity in the cortex and hippocampus of PSAPP mice returned to normal levels after administration of CAE. The alteration in the kinases such as ERK MAP kinase, JNK, p70S6 kinase and other cellular biomarkers such as STAT5, STAT and CREB in the PSAPP mice was reversed by the CAE administration. The level of glutamate receptors and AMPA receptors in hippocampus and cortex of PSAPP was also found to be altered in PSAPP mice. Administration of CAE diminished the alteration in glutamate and AMPA receptor subunits. The study reveals that by acting on various causal pathways involved in this multifactorial disorder, the CAE holds a promising therapeutic value that should be exploited in the clinical world.
Acknowledgement: Alzheimer's Association grant (NIRG-08-91816)

PS 528 CHARACTERIZATION OF LYMPHOCYTE SUBSETS IN PERIPHERAL BLOOD OF HEALTHY, OBESE, AND TUMOR-BEARING DOGS.

M. Ko[1], Q. Peng[1], A. Gonzales[2], E. McCandless[2], M. Aleo[2], R. McCall[2], S. Kamerling[2], Q. Zong[1], D. Sace[1], R. Yafawi[1], J. Wang[1] and A. John-Baptiste[1]. [1]*Drug Safety, Research & Developement, Pfizer, San Diego, CA and* [2]*Animal Health, Pfizer, Kalamazoo, MI.* Sponsor: W. Huang.

Dog is an important animal model for studying various diseases such as cancer, allergy, auto-immune disorders, and infection. Monitoring changes in peripheral blood lymphocyte subsets can be a valuable experimental tool for understanding disease mechanisms and developing new immunotherapeutic treatments. The purpose of this study was to characterize lymphocyte subsets of peripheral blood in healthy, obese, and tumor-bearing dogs to assess their usefulness as biomarkers for studying various canine diseases. Peripheral blood samples from healthy, purposebred beagles (n=30), beagles with mammary gland tumors (n=15), and obese beagles (n=47) were collected for immunophenotyping. The blood samples were stained for T cells, B cells, Helper T cells, and Cytotoxic T cells and analyzed by flow cytometry. The percentage of T cells in the peripheral blood was significantly different among the three groups, but the proportion of B cells was only significantly different between healthy and tumor dogs. Within the T-lymphocyte population, there was no statistical difference in CD4+ Helper T cells among the three groups, but the percentage of CD8+ Cytotoxic T cells was significantly lower in the obese dogs compared to healthy animals. When CD4:CD8 ratios were calculated, the ratio was significantly higher for the obese dogs compared to the healthy dogs. Both tumor and obese groups showed significantly lower percentage of CD4+CD8+ compared to healthy group. Without statistical significance, both tumor and obese dogs had higher proportion of CD4-CD8- populations than the healthy dogs. The significance of CD4CD8 double positive and double negative populations are unknown at this time. Finally, serum IgE levels correlated with % IgE+ B cells in eighteen healthy dogs (R2 = 0.654). In summary, a variety of differences found in lymphocyte subsets of disease animals suggests immunophenotyping as a powerful tool in investigating underlying mechanisms of disease.

PS 529 INVESTIGATION, OPTIMIZATION, AND VALIDATION OF MINIPIG IMMUNOPHENOTYPING BY FLOW CYTOMETRY.

A. Shen and S. Sokolowski. *Drug Safety R&D, Pfizer, Inc., Groton, CT.* Sponsor: J. Colangelo.

Accurate assessment of immunophenotyping in minipigs has proven to be very challenging due to the lack of commercially available antibodies. This study provided optimization and validation of methods and reagents to measure lymphocyte subsets in the peripheral blood of Göttingen minipigs. Red blood cell lysis procedures were compared to achieve optimal separation of cell populations while maintaining appropriate antibody labeling. Gamma delta T lymphocytes, T helper/inducer lymphocytes, cytotoxic/suppressor T lymphocytes, B lymphocytes and NK cell populations in peripheral blood were quantitated by flow cytometry. Reference ranges were determined and reported as percent of total lymphocytes and absolute cell count per microliter. Results obtained for twenty male and female Göttingen minipigs, aged 2.5 to 4.5 months old, (~40% gamma delta T cell, 23% T helper, 13% cytotoxic T, 10% B cell, 2.5% NK cells) were similar to those reported in the literature. Evaluation of assay precision for all parameters was acceptable with a coefficient of variation less than 10%. Sample stability testing showed whole blood to be stable for 48 hours when stored under refrigerated condition. All stained samples were shown to be stable for at least 48 hours. This study allowed the development and validation of immunophenotyping protocols using commercially available antibodies that accurately measure lymphocyte subsets in minipig peripheral blood.

PS 530 IGE POSITIVITY IN B LYMPHOCYTES: A VALUABLE BIOMARKER FOR DIAGNOSIS OF CANINE ALLERGY.

Q. Peng[1], M. Ko[1], A. Gonzales[1, 2], E. McCandless[1, 2], Q. Zong[1], F. Sace[1], R. Yafawi[1], J. Wang[1] and A. John-Baptiste[1]. [1]*DSRD, Pfizer, San Diego, CA and* [2]*Animal Health, Pfizer, Kalamazoo, MI.* Sponsor: G. Stevens.

Allergen-specific IgE increase in blood indicates allergy and hypersensitivity. However, specific IgE concentration is neither an easy task to perform nor an accurate predictor. Therefore, IgE positive B cells are explored as an alternative for diagnosis and other potential applications by flow cytometry. The goal is to investigate whether IgE positivity in B cells increases in canine allergy. First, specificity of anti-dog IgE antibodies was tested. Two commercial available anti-dog IgE antibodies were performed. Antibody from company A was tested to be 1~4% positivity in B lymphocytes among 4 healthy beagle dog; Antibody from company B was tested to be high 20% or more than 40% positivity in B lymphocytes among the same 4 beagle dogs, which is not expected since majority of mature B cells should be IgG producing cells. The company B anti-dog IgE showed cross reactivity (2.9-5.5%) among CD21(-) lymphocytes while company A anti-dog IgE antibody confirmed to be negative among the CD21(-) lymphocytes, which indicates its specificity. Second, IgE positivity in B cells of 12 beagle dogs (6 flea infected and 6 healthy beagles) were tested. The 12 dog blood samples were collected in two different dates (one week apart) and tested in three times to get a mean value of the IgE positivity in B cells. The result showed that IgE positivity in B lymphocytes (8.8% by mean value) varied from 4.1% to 11% among allergic dogs. IgE positivity in B lymphocytes showed from 0.38% to 2.9% in variation with a mean value of 1.3% among the healthy dogs. The P value in unpaired TTest was <0.001, which indicated significant difference. In conclusion, IgE positivity in B lymphocytes seemed to be a valuable biomarker for diagnosis of canine allergy, especially for flea related allergy.

The IgE positivity in B cells can also be applied as a biomarker for monitoring allergic reaction, which can be induced by medication that causes such an allergy, specifically in topical drug application.

PS 531 INFLUENCE OF A MIXTURE OF FIVE PESTICIDES ON THE REPRODUCTIVE SYSTEM OF FEMALE SPRAGUE-DAWLEY RATS.

V. M. Pacotto[1], M. T. Guerra[2], J. V. de Camargo[1] and C. S. Franchi[1]. [1]*Pathology, Sao Paulo State University - UNESP - Medical School, Botucatu, SP, Brazil and* [2]*Graduate Program in Cell and Structural Biology, State University of Campinas, Campinas, SP, Brazil.* Sponsor: S. Cohen.

Environmental contaminants, such as some individual or mixed pesticides, may compromise the endocrine and reproductive system of mammals and or their descendants and are considered in the class of endocrine disruptors. This study, approved by the local Committee for Ethicts in Animal Experimentation, aimed to evaluate the effects of a dietary mixture of five pesticides (dieldrin, dicofol, endosulfan, dichlorvos and permethrin) on the reproductive system of female rats. Six-week-old female Sprague-Dawley rats were allocated to three groups: Group 1: basal control diet; Group 2: mixture of pesticides at their respective NOEL: dichlorvos (0.23 mg/kg/day), dicofol (0.5 mg/kg/day), dieldrin (0.05 mg/kg/day), endosulfan (0.7 mg/kg/day), permethrin (5 mg/kg/day); Group 3: the same, at the LOEL levels: dichlorvos (2.3 mg/kg/day), dicofol (2.1 mg/kg/day), dieldrin (0.05 mg/kg/day), endosulfan (3.8 mg/kg/day), permethrin (25 mg/kg/day). Vaginal smears were collected daily from the 8th to the 10th week to verify the phases of the estrous cycle; the animals were successively euthanized once they entered the estrous, what occurred within the 10th to the 12th week of experiment. Normal oestrous cyclicity was maintained and endometrial thickness, and progesterone and LH serum levels did not differ among groups. Both mixture-fed groups showed increased mean levels of serum FSH, but only the Group 2 differed significantly from control. Animals fed the mixture at LOEL levels(Group 3) developed signs of systemic influence of the pesticides, as reduced gain of body weight (-20%), increased relative liver weight (+10%) and centrilobular hypertrophy (2/10 animals). These data suggest that exposure to mixtures of these five pesticides influence the female rat homeostasis even when the mixtures use concentrations of the components at a non-effect level. FAPESP Proc. 06/60506-1.

PS 532 BIOLOGICAL PATHWAY ANALYSES OF HEPATOTOXICANT-INDUCED CHANGES IN HEPATIC GENE EXPRESSION IN CHIMERIC PXB-MOUSE® WITH HIGHLY HUMANIZED LIVER.

S. Nagatsuka[1], D. Hynes[1], S. Ninomiya[1], M. Kakuni[2], C. Tateno-Mukaidani[2], T. Shimada[2] and Y. Yamazoe[3]. [1]*R&D, Sekisui Medical Co., ADME & Toxicology . Research Institute, Tokai-mura, Ibaraki, Japan,* [2]*PhoenixBio Co., Higashi-Hiroshima, Hiroshima, Japan and* [3]*Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi, Japan.* Sponsor: T. Miyaoka.

Drug-induced liver injury (DILI) in humans is one of the leading causes of withdrawal of a newly developed drug, and is difficult to evaluate by classical *in vivo* toxicity studies using experimental animals. Toxicogenomic approaches, which examine early cellular responses that are considered to be more robust against variations due to species difference, have been employed using *in vivo* (experimental animals) or *in vitro* (human hepatocytes) systems. In the present study, chimeric PXB-mice, in which more than 70% of hepatic parenchymal cells are replaced by human hepatocytes, were employed for the toxicogenomic analyses of hepatotoxicants. PXB-mice mimic human-type drug metabolism and, therefore, have the potential to explain the differences between rodent and human livers, and also between *in vivo* and *in vitro* responses of human hepatocytes. By using 16 different hepatotoxicants (acetaminophen, amiodarone, diclofenac, d-penicillamine, flutamide, erythromycin, valproate, sulindac, indomethacin, perhexilene, methyldopa, amitriptyline, tamoxifen, acetylsalicylic acid, methotrexate, demeclocycline) and seven non-hepatotoxicants, we analyzed changes in hepatic gene expression in PXB-mice. These drugs were orally administered to PXB-mice three-times daily at high doses (ca. 20% of reported LD_{50}), followed by hepatic total RNA preparation and gene expression analyses using Affymetrix GeneChip® Human Genome U133 plus 2.0 Array. Various biological pathways related to DILI (aryl hydrocarbon receptor, NRF2, NFκB, HIF1, EIF2A, TGFβ, apoptosis, cell cycle, CAR/PXR, fatty acid biosynthesis, β-oxidation, cholesterol biosynthesis, bile acid biosynthesis etc.) were selected for the analyses of changes in hepatic gene expression. The relationship between DILI and these biological pathways will be discussed.

PS 533 THE EFFECTS OF CILOSTAZOL ON MOUSE OOCYTE MEIOTIC MATURATION AND MORPHOLOGY.

A. M. Taiyeb[1], D. Kraemer[1], V. Fajt[1] and C. M. Sayes[1, 2]. [1]*Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX and* [2]*Interdisciplinary Program in Toxicology, Texas A&M University, College Station, TX.*

Cilostazol (CZL) is a phosphodiesterase 3 enzyme inhibitor (PDE3-I) that has been used to treat patients with Intermittent Claudication disease. CZL has also the ability to decrease the cellular adenosine (Ado) uptake that antagonizes the many side effects of other PDE3-Is and may present CZL as a potential contraceptive. In order to study the possible contraceptive effect of CZL on mammals, immature Swiss albino mice were injected with 5IU PMSG and oocytes were retrieved after 48hr. Denuded oocytes were cultured with 10 different CZL concentrations (T1: 0.00 [control group], T2: 0.264, T3: 0.528, T4: 1.057, T5: 2.114, T6: 4.229, T7: 8.458, T8: 16.916, T9: 33.832, and T10: 67.664μM CZL) and assessed for the maturational status and normal morphology after 24 and 48hr of incubation. CZL at concentrations of T4-T10 (24hr) and concentrations of T4-T8 (48hr) significantly arrested the oocytes at the germinal vesicle (GV) stage compared to control ($P< 0.0001$). The decline in the total percentage of oocytes arrested at GV stage after 24hr for the concentrations of T9-T10 (72.3 and 68.03%) versus T 6-T8 (85.36, 88.13, and 82.38%) was due to the higher degenerative rates of T8-T10 on oocytes versus T1-T7 ($P<0.0005$). CZL at concentrations T9-T10 after 48hr had more toxicological impact on oocytes morphology than on arresting meiotic maturation. The insignificant meiotic inhibition of T9-T10 after 48hr is due to the high degeneration rate when compared with the same concentrations for 24hr and T1-T7 ($P<0.0001$). It is concluded that CZL is effective in arresting mouse oocyte meiotic maturation. This inhibitory action of CZL may be due to the synergistic effect of PDE3, and Ado uptake inhibitions. The high degenerative rate of oocytes that was observed at higher concentrations of CZL may be due to depriving the oocytes of adenosine and inhibiting their biochemical activities. Our study shows that CZL is a potential contraceptive agent that is appropriate for further study.

PS 534 3D LIVER CO-CULTURES MAINTAIN PHASE I, II, AND III METABOLISM ACTIVITY IN LONG-TERM CULTURE.

J. Llamas Vidales[1], L. New[1], C. I. Pearson[1], A. H. Roter[2], B. A. Naughton[1] and D. R. Applegate[1]. [1]*R&D, RegeneMed, Inc., San Diego, CA and* [2]*Entelos, Inc., Foster City, CA.* Sponsor: R. Thomas.

Pharmaceutical companies require more accurate toxicity screening of drug candidates earlier in the discovery process in order to increase success at later stages of drug development. However, toxicity screening has typically relied upon in vitro primary hepatocyte cultures, which lose many important liver functions, including P450 activity, Phase II drug metabolism, and hepatobiliary transport, within a few days of culture. Organotypic three-dimensional liver co-cultures (3DLC) have been shown to maintain liver function for several months in culture, and can provide a better tool for liver toxicity screening. Male rat 3DLC showed similar baseline gene expression of most Phase I, II, and III drug metabolism genes (such as Cyp3a, Cyp4a, Gst2a/3a, Ugt1a1, Ugt2b, Abcc1, Abcc2, and Abcc4) to intact liver. Exceptions include P450s, such as Cyp1a1, which was expressed at a higher level in 3DLC, and Cyp2b1, which was expressed at a lower level in 3DLC. Phenobarbital, a known CAR/PXR agonist, elicited similar gene expression induction patterns between 3DLC and in vivo liver over 18 days of exposure, even among those P450s which had lower or higher baseline gene expression in 3DLC. P450 expression response to inflammatory compounds in 3DLCs was also similar to in vivo. Moreover, the activity of P450s Cyp1a1, Cyp3a, and Cyp4a was shown to remain inducible in 3DLC by AhR, CAR/PXR, and PPARα agonists, respectively, over 70 days of culture. These and previous studies show that 3DLC are a reliable in vitro model for the assessment of long-term effects of chemical compounds on in vivo liver function, including toxicity, P450 induction and function, and biliary transport.

PS 535 ATP-BINDING-CASSETTE TRANSPORTER KNOCKOUT RAT MODELS.

I. D. Carbery, L. C. Little, E. J. Weinstein and X. Cui. *SAGE, Sigma-Aldrich, St. Louis, MO.*

Animal models are an essential element of drug development and toxicity assessment. Rats are preferred over mice for their closer resemblance to human anatomy and physiology and their larger size. Until recently, knockout rats were not readily available. To knockout genes in the rat, Zinc Finger Nucleases (ZFNs) are injected into one-cell rodent embryos to introduce site-specific modifications on the chromosome, leading to the disruption of gene function (1). ATP-binding-cassette

(ABC) transporters actively transport xenobiotic compounds in vivo, but are also involved in multidrug resistance of tumors. The study of these transporters is relevant to the development of chemotherapeutic agents. Here we report the successful creation of the suite of ABC transporters knockout models Mdr1a, Mrp1, Mrp2, and Bcrp using ZFN technology. Homozygous knockout animals were confirmed by the lack of respective proteins in Western Blots. Mrp2 homozygous animals are obviously jaundice after birth, a phenotype seen in TR- rats and patients with the Dubin-Johnson syndrome, all of which have mutations that disrupt the Mrp2 gene. All models are being tested in pharmacokinetics studies.

PS 536 PATHOLOGY OF A BROWN-NORWAY RAT MODEL OF AMODIAQUINE-INDUCED LIVER INJURY.

H. C. Workman[1], J. Li[2], P. Cai[3], M. Odin[1], M. David[1] and J. Uetrecht[3, 4]. *[1]Non-Clinical Safety, Hoffmann-La Roche, Nutley, NJ, [2]Immunotoxicology, Bristol-Myers Squibb, New Brunswick, NJ, [3]Pharmacy, University of Toronto, Toronto, ON, Canada and [4]Medicine, University of Toronto, Toronto, ON, Canada.*

Rationale Amodiaquine (AQ), an anti-malarial drug still available in some countries, is reported to cause idiosyncratic drug induced liver injury (I-DILI) in people. Lesions in people are poorly described; findings usually include mononuclear cell infiltration, Kupffer cell activation (occasionally containing cytoplasmic pigment), hepatocellular necrosis, bile duct hyperplasia and fibrosis. The underlying mechanism causing the pathology of DILI is not well understood nor is an animal model for this type of DILI currently available. We used the BN rat, prone to develop immune-type response, to help further characterize the pathology of this disease. Experimental procedures Male Brown-Norway (BN) rats were treated with AQ at 62.5 mg/kg/day PO for 42 days and a second study for 49 days was repeated. Histopathology included hematoxylin and eosin (H&E), immunohistochemistry and special histochemical stained sections of a complete tissue set. Results Liver findings included scattered individual Kupffer cell increase and activation as well as small microgranulomas of activated macrophages admixed with few lymphocytes. Large aggregates of macrophages with iron positive intracytoplasmic pigment showed a bridging pattern, primarily around portal tracts and occasionally random. Other findings included (scattered) single hepatocellular necrosis/apoptosis and bile duct hyperplasia. Spleen findings included macrophage activation in the red pulp, follicular hyperplasia and variable depletion of the marginal zone in the white pulp. Other findings were those reported with the BN rat strain. Conclusions The pathology reported in this AQ BN rat model exhibited similarities with that reported in AQ-induced liver injury in patients. Additional work is needed to further characterize this model and understand the immunological mechanisms therein.

PS 537 ALTERED BEHAVIOR, BODY WEIGHT, AND BRAIN CHEMISTRY IN PAK5-/-, PAK6 -/-AND PAK5-/-/PAK6-/- MICE.

M. A. Furnari[1], M. L. Jobes[1], T. Nekrasova[2], A. Minden[2] and G. C. Wagner[1, 3]. *[1]Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ, [2]Susan Lehman Cullman Laboratory for Cancer Research, Rutgers University, Piscataway, NJ and [3]Psychology, Rutgers University, Piscataway, NJ.*

PAK5 and PAK6 are protein kinases highly expressed in brain. We observed that PAK5 and PAK6 single knockout mice and PAK5/PAK6 double-knockout mice(DKO) displayed altered behavior and that PAK6 single knockout mice gained significantly more weight than other genotypes, including wildtypes(WT). Based on this work, further investigation of these genotypes was done. Mice of each genotype were given free access to run wheel exercise or placed in cages without run wheels for a total of 74 days. Body weight, fluid and food consumption were measured weekly. PAK5 and PAK6 single knockouts, DKOs, and WT mice were treated with a single dose of amphetamine (50mg/kg) and sacrificed 72 hours later. The striatum was dissected and assayed for dopamine(DA), serotonin(5-HT) and their metabolites dihydryoxyphenylacetic acid(DOPAC), homovanillic acid(HVA), and 5-hydroxyindoleacetic acid(5-HIAA) by high pressure liquid chromatography(HPLC). While run wheel mice consumed significantly more food, they weighed less than non-run wheel mice. In addition, although PAK6 knockout mice consumed the same amount of food as wildtype mice, they were significantly heavier regardless of run wheel condition. These data suggest that PAK6 may be involved in the regulation of body fat deposition. Dopamine significantly lower in all genotypes that received amphetamine while DOPAC, 5-HT, HVA, and 5-HIAA were not different from saline treated controls. There was no significant difference in any neurotransmitter or metabolite between saline- and amphetamine-treated groups in WT, PAK5 knockouts and DKs. The PAK6 knockout mice treated with amphetamine did show a significant decrease in dopamine compared to controls. These data suggest that PAK6 may be involved in the regulation of body fat deposition and that the PAK6 mice have an increased sensitivity to the neurotoxic effects of amphetamine. Supported in part by ES005022 &T32ES007148.

PS 538 THE TOXICITY OF METHIMAZOLE IN THE MOUSE OLFACTORY MUCOSA IS AT LEAST PARTLY MEDIATED THROUGH TARGET-TISSUE METABOLIC ACTIVATION BY CYP2A5.

J. Gu[1], F. Xie[1], X. Zhou[1], M. Genter[2] and X. Ding[1]. *[1]Wadsworth Center, New York State Department of Health, Albany, NY and [2]Department of Environmental Health, University of Cincinnati, Cincinnati, OH.*

The anti-thyroid drug methimazole (MMZ) can cause severe, tissue-specific toxicity in mouse olfactory mucosa (OM). The toxicity of MMZ is mediated through its reactive intermediates (sulfenic and sulfinic acids), formed presumably by a sequential activation of MMZ, by P450 and flavin monooxygenases (FMO). The specific P450 enzymes responsible for MMZ metabolic activation have not been identified, and it is unclear whether target-tissue metabolic activation is essential for toxicity. The aims of the present study were to determine whether CYP2A5, one of the most abundant P450 enzymes in the mouse OM, is involved in MMZ metabolic activation, by comparing Cyp2a5-null with wild-type (WT) mice, and whether hepatic microsomal P450 enzymes, including CYP2A5, are essential for MMZ-induced OM toxicity, by comparing liver-Cpr-null (LCN) mice, which have little P450 activity in hepatocytes, with WT mice. Our results showed that the loss of CYP2A5 expression did not alter systemic clearance of MMZ (at 50 mg/kg, i.p.); but it did significantly decrease the rates of MMZ metabolism in the OM, while olfactory FMO expression was not decreased. MMZ induced depletion of non-protein thiols, as well as pathological changes, in the OM of WT mice; the extent of these changes was much reduced in the *Cyp2a5*-null mice. Thus, CYP2A5 plays an important role in mediating MMZ toxicity in the OM. In contrast, the rate of systemic clearance of MMZ was significantly reduced in the LCN mice, compared to WT mice, whereas the MMZ-induced OM toxicity was not prevented. Therefore, hepatic P450 enzymes are essential for systemic MMZ clearance, but they are not required for MMZ-induced OM toxicity. We conclude that the tissue-specific toxicity of MMZ is mediated by target tissue metabolic activation, and the reaction is at least partly catalyzed by CYP2A5 in the OM. (Supported in part by NIH grant ES007462)

PS 539 MODELS OF ACUTE LUNG INFLAMMATION IN NON HUMAN PRIMATES: LPS, MECHANICAL VENTILATION, PSEUDOMONAS AERUGINOSA, AND CIGARETTE SMOKE.

R. W. Spindle, M. Doyle-Eisele, O. Denise, J. Tipper, K. Harrod, R. Jaramillo and J. McDonald. *LRRI, Albuquerque, NM.*

Non-human primates (NHP) may serve as an important translational research tool for the evaluation of mechanisms of disease pathogenesis and efficacy of new therapeutics. We have defined the pathogenesis of several acute lung injury models in NHPs, including inhaled lipopolysaccharides (LPS), instilled Pseudomonas Aeruginosa (P. Aeruginosa), inhaled cigarette smoke (CS) and mechanical ventilation. For each animal model, inflammation is assessed in bronchoalveolar lavage (BAL) collected prior to the initial challenge and prior to necropsy. For LPS, animals received a single inhalation exposure (5 mg/m3) by face mask 24 hrs prior to necropsy. Significant increases in neutrophils, IL5, IL8 and TNFα were observed in BAL compared to control animals. This inflammation was reduced by treatment with inhaled budesonide (0.7 mg/kg). To evaluate the ability to potentiate the LPS injury to represent an acute lung injury model, the same LPS dose was administered to animals approximately 24 hours prior to being placed on an Intensive care unit (ICU) ventilator. Animals were held at high-peak pressure ventilation for 3-6 hours under systemic anesthesia. Inflammation and protein measured in BAL was elevated immediately post ventilation compared to the control. These results were greater than ventilation or LPS alone. For P. Aeruginosa, animals were instilled with 10^9 colony forming units or saline on Day 0 and were observed out through Day 4. Neutrophil infiltration and pneumonitis was observed compared to control animals. Inflammation in NHPs exposed to CS was confirmed after exposure for up to 4 weeks of exposure. Each of these models identifies new tools for the assessment of acute inflammation in NHPs.

PS 540 EVALUATING THE CELLULAR CHANGES IN BLEOMYCIN-INDUCED PULMONARY FIBROSIS MODELS: IT VS INHALATION.

M. Doyle-Eisele, R. W. Spindle, A. Gigliotti and J. D. McDonald. *LRRI, Albuquerque, NM.*

Intratracheal (IT) bleomycin sulfate (BS) is one of the most commonly used animal models for pulmonary fibrosis (PF). This model regularly causes patchy fibrosis in the lung. While IT models do not elucidate the human condition, they are representative of many of the clinical and subclinical PF characteristics. Inhalation

(INH) BS causes more diffuse fibrosis in the lung. Similar to other inflammatory models (i.e. LPS), INH administration has demonstrated a more distributed pattern of injury which may closely mimic the human condition. Both models cause 7-10 days of pneumonia-like symptoms that resolve through 14-35 days for the onset of PF. In this study, we compared the time-course of cellular mediators (PMN, collagen, IL1beta, MCP-1, TNFalpha, IL4, IL6, IL10, GROKC, RANTES and IFNgamma) associated with the manifestation of PF in male, F344 rats. Animals were examined for in-life clinical signs, gross and microscopic pathology, and changes in cellular mediators at the following endpoints: 5, 7, 10, 14, 21, 28 and 35 days post BS administration. Rats received 3.5-4.0 mg/kg BS on Day 0 and were observed through 35 days. In these rat models, PMN, MCP1, IL1beta, IL6, GROKC, and soluble collagen upregulation (or release) trends were similar between models. By Day 21 inflammation as measured by PMN, MCP1, IL1beta, GROKC, and IL6 returned to baseline levels. TNFalpha was consistently greater than baseline through 14 days and then spiked again at 35 days. In parallel to the inflammatory marks, IL10, IL4, and RANTES were suppressed compared to baseline levels throughout the disease progression. Fibrotic lesions were grossly observed beginning at 14 days – data supported by increases in lung collagen. Soluble collagen was 2-3X baseline beginning at 5 days and stayed elevated through 35 days post BS administration. Additional endpoints of interest under evaluation include αSMA, CTGF, MMP7, LDH, and TGFbeta.

PS 541 THE PATHOPHYSIOLOGY OF PROGRESSIVE EXPOSURE TO TOBACCO SMOKE IN A RODENT MODEL OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD).

L. Wang[1], S. Bolton[2] and K. E. Pinkerton[1]. [1]*Center for Health and the Environment, University of California, Davis, Davis, CA and* [2]*Safety Assessment, AstraZeneca R&D, Loughborough, United Kingdom.*

Background: COPD is a leading cause of morbidity and mortality worldwide, however, we are still far from understanding the complexity and progressive development of this devastating disease. Objectives: To understand the interactive roles of pulmonary inflammation, lung physiology and lung tissue remodeling in disease progression in a rat model of COPD. Methods: Spontaneous hypertensive rats were exposed to tobacco smoke (TS) (90 mg/m3) or filtered air (FA) for up to 12 weeks. Timepoints of 4, 6, 8, 10 and 12 weeks for necropsy were chosen to define the pathophysiological changes in the lung. Results: Bronchoalveolar lavage (BAL), cell differential, histopathology and lung function tests were performed. Rats exposed to TS demonstrated an enhanced BAL, airway and alveolar inflammatory responses reflected by significant increases in macrophage, neutrophil and lymphocyte number as well as inflammatory cytokines compared to FA at 4, 6, 8, 10 and 12 weeks. The degree of inflammation increased temporally and peaked between week 6 and week 8. Airway obstruction was noted by a significant elevation in central airway resistance, tissue damping and a reduction in total respiratory compliance due to TS exposure. Central airway resistance demonstrated a decrease at 4 weeks, an increase at 8 weeks, peaking at 10 weeks and remaining elevated at 12 weeks. Conclusions: These findings suggest a persistent, but dynamic airway inflammatory response and airway obstruction in the rat model of COPD. Airway inflammation alone can not explain the variance in physiological lung function impairment observed in the TS rats, implying tissue repair and airway remodeling may also contribute to the airflow limitation. This rat model of COPD could address critical questions in the mechanistic progression of inflammation and tissue repair in COPD, which could have implications for future therapy and new drugs. Funded by AstraZeneca and California TRDRP

PS 542 USE OF MOUSE MODEL AS AN EARLY SCREENING TOOL FOR PDE4 INHIBITOR-INDUCED GASTROINTESTINAL TOXICITY.

C. Chen, H. Sexton, K. Jarnagin, X. Fan, W. Bu, A. Wu, Y. Freund and S. Chanda. *Toxicology, Anacor Pharmaceuticals, Palo Alto, CA.*

To allow rapid assessment of PDE4 inhibitors-related gastrointestinal (GI) toxicity, an early in vivo screening model was developed in mice or rats. PDE4 inhibitors have been reported to cause toxicities in various animal species including dogs, rats, minipigs, and ferrets. However, limited toxicity studies have been conducted in mice. Animals were dosed with candidate compounds for 5 consecutive days. Toxicity evaluation included clinical observation, body weight, gross observation/GI measurement at necropsy, hematology and fibrinogen analyses. In the mouse, stomach enlargement was the primary GI finding, which correlated with fibrinogen elevation. In the rat, GI effects were most severe in the jejunum and ileum and the GI dilation was highly correlated with increases in neutrophils, white blood cells and fibrinogen. In these two screening models, results revealed that rat NOAEL for GI toxicity was 1/2 or 1/3 of that observed in mice treated with the same test compound. The mouse model is considered to be a useful early screening tool based on the following advantages: 1) milligram quantities of test article are needed, 2) stomach enlargement and fibrinogen elevation are considered as the primary indictors for PDE4-related GI toxicity. This mouse model was used to investigate whether the PDE4 inhibitor-induced GI toxicity was associated with local or systemic exposure. Mice were dosed once daily for 5 days either via subcutaneous (sc) injection or oral gavage. Results indicated that the stomach enlargement was not significantly reduced via sc injection compared to oral dosing, and the systemic exposure of test compound was comparable between oral and sc dosing routes. The results suggested that the PDE4-induced GI toxicity is correlated with systemic exposure and compound affinity. In conclusion, the mouse toxicity screening model is considered as a useful in vivo tool for the screening PDE4 inhibitors at an early stage of drug development and was successfully used to evaluate GI toxicity of selected PDE4 inhibitors.

PS 543 EFFICACY OF PHARMACEUTICAL DRUG COCKTAILS FOR TREATING CHLORINE VAPOR INDUCED CUTANEOUS LESIONS.

J. Rhone[1], J. L. Plahovinsak[1], E. M. Benson[1], R. C. Hamilton[1], F. M. Reid[1], J. F. Dillman[2] and J. S. Graham[2]. [1]*Biomedical Research Center, Battelle, Columbus, OH and* [2]*U.S. Army Medical Research Institute of Chemical Defense, Aberdeen, MD.*

Chlorine is an industrial chemical that potentially causes cutaneous burns. The unforeseen exposure to highly hazardous toxic industrial chemicals has presented the need to develop animal models for evaluating promising therapeutic compounds against these chemicals. Battelle's Biomedical Research Center and the U.S. Army Medical Research Institute of Chemical Defense have aligned research efforts for efficacy testing of FDA-approved candidate drugs to promote healing in cutaneous chlorine vapor (CLV) injuries. A CLV exposure system was previously fabricated and characterized to induce superficial dermal (SD) lesion sites on the ventral abdomen of Yorkshire-cross weanling pigs. Sites were exposed to undiluted chlorine vapor (2.9 g/L) for thirty minutes following the application of a simulated sweat soaked filter paper. This model was used to assess the efficacy of topical anti-inflammatory compounds: diclofenac sodium and clobetasol propionate with two anti-cytokine drugs post-exposure. A third treatment group received topical treatments only. Lesion assessments were conducted on Study Day 0 (infrared imagery only), and Study Days 2, 3, and 7 to include digital photographs, wound size measurements, modified Draize scoring, and non-invasive bioengineering methods (reflectance colorimetry and infrared imagery). Histopathologic evaluations were performed for wound healing parameters (re-epithelialization, dermal necrosis, and fibroplasia) on Study Day 7. The administration of Enbrel and Kineret without topical anti-inflammatories resulted in reduced lesion size, lower mean modified Draize scores, and reduced erythema on Study Days 2 and 3. Complete re-epithelialization was observed in the no treatment sites suggesting that CLV induced SD lesions may heal without treatment by day 7 post-exposure.

PS 544 EFFICACY OF PHARMACEUTICAL DRUG COCKTAILS FOR AMELIORATION OF CUTANEOUS SULFUR MUSTARD INJURIES.

F. M. Reid[1], J. L. Plahovinsak[1], M. D. Etheridge[1], J. Rhone[1], J. F. Dillman[2] and J. S. Graham[2]. [1]*Biomedical Research Center, Battelle, Columbus, OH and* [2]*U.S. Army Medical Research Institute of Chemical Defense, Aberdeen, MD.*

The vesicant agent, sulfur mustard [bis(2-chloroethyl)sulfide; SM], remains a threat to warfighters and civilians worldwide via acts of war and terrorism. Battelle's Biomedical Research Center (BBRC), and the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) have aligned research efforts for efficacy testing of candidate drugs to promote wound healing of injuries induced by SM. Recent work has focused on topical anti-inflammatory compounds: diclofenac sodium and clobetasol propionate. Using an established SM dermal weanling swine model, this study evaluated the efficacy of FDA-approved drugs in combination with topical anti-inflammatory compounds for wound healing in superficial and deep dermal cutaneous injuries. Two different anti-cytokine drugs were administered to animals post- exposure in combination with the topical anti-inflammatory compounds. A third treatment group received the topical compounds only. Treatment was initiated at 2 hours post exposure. Lesion assessments were conducted on Study Days 0 (infrared imagery only), 2, 3, and 7. Assessments included digital photographs, wound size measurements, modified Draize scoring, reflectance colorimetry, and infrared imagery (day 0 only). Histopathologic evaluations were performed for wound healing parameters (re-epithelialization, dermal necrosis, and fibroplasia) on Study Day 7. The administration of both Enbrel and Thalidomide showed promise in healing HD-induced lesions particularly when used with both topical anti-inflammatory drugs. This was confirmed histopathologically by decreased necrosis and increased re-epithelialization. Follow on efforts will examine additional FDA-approved drugs in combination with topical anti-inflammatory drugs as determined from the canonical pathway analysis.

PS 545 BEHAVIORAL EFFECTS OF REPIN IN MICE.

W. K. Rumbeiha and J. Nunez. *Michigan State University, East Lansing, MI.*

Chronic ingestion of yellow star thistle (Centaurea solstitialis) or Russian knapweed (Centaurea repens) causes equine nigropallidal encephalomalacia (NPE) aka equine parkinsonism. Clinical signs and pathology of equine NPE were first described by Cordy in 1954. They are always sudden in onset and include depression, with peculiar stances and gait abnormalities. Dystonia of facial, lip and tongue muscles renders them unable to eat or chew normally. Affected horses succumb to starvation and dehydration. Repin, a natural sesquiterpene lactone derived from the two poisonous plants is the presumed cause of NPE. Parkinson's disease is characterized by movement and behavior disorders. The laboratory is developing a novel repin-induced model of Parkinson's disease in mice. The purpose of this investigation was to determine the behavioral effects of repin in C57BL/6j male mice. Three experiments were conducted: acute single dose study, chronic 28 day study, and chronic 109 day study. For the acute study, groups of mice received either the vehicle or 5 mg repin/kg bw subcutaneously (SC) and behavioral tests were conducted between 6-9 hrs post dose. For the 28 day study, mice received either vehicle or repin at 5 mg/kg bw 3 times weekly SC for 28 days. For the 109 day study, mice received either vehicle or repin at 2.5 mg repin SC for the first 50 days and increased to 5 mg/kg bw repin SC for the reminder of the study. Behavioral tests conducted included the elevated plus maze, the open field maze, the water maze, the balance beam, and social interaction. In both acute and chronic studies repin significantly reduced both outer and inner crossings in the open field maze ($p<0.05$). Repin also consistently and significantly reduced time in the open, as well as visits to the three thirds of the elevated plus maze (($p<0.05$). On the balance beam, repin caused a statistically significant increase in back leg slips compared to control. However, this effect was not consistent. These results suggest that repin is anxiogenic and causes some motor effects in mice. More refined motor tests are recommended.

PS 546 INTRAVITREOUS INJECTION-RELATED RESPONSES AND THEIR MANAGEMENT IN NON-HUMAN PRIMATE - A CASE STUDY.

T. T. Lam, J. Seeman and B. J. Christian. *Covance, Madison, WI.*

There is an increasing need for IVT injection studies in animals. In this case study, we followed the responses and their management after IVT injection in 36 (18 males and 18 females) cynomolgus monkeys. All procedures and treatments were performed with the approval of the Institutional Animal Care and Use Committee of Covance. Both eyes of each animal were given IVT injections of 50 µL once every 2 weeks. A 0.1% betadine solution followed by rinsing with saline was used to prepare the eyes for injection. For the 1st IVT injection, no prophylactic treatment for ocular discomfort was given. In the afternoon of the dosing day, none of the males but 10 of the females showed bleopharospasm, and eye squinting. All females were treated with intramuscular injections of 2 mg/kg flunixin and 0.05 mg/kg buprenorphine and 2 of them required further 0.05 mg/kg buprenorphine injection the morning after. No subsequent treatment was needed. For the 2nd injection, all animals were given prophylactic 2 mg/kg flunixin prior to IVT injection and 0.05 mg/kg buprenorphine immediately and at 6 hours after IVT injection. In the afternoon, 10 of 18 males and 18 of 18 females showed bleopharospasm, and eye squinting. All males were given 2 mg/kg flunixin and an additional dose of 0.05 mg/kg buprenorphine. All females were given 0.05 mg/kg buprenorphine only. In the morning after the 2nd injection, 2 of the 18 females showed eye squinting and required one dose of buprenorphine. No subsequent treatment was needed. From the 3rd IVT injection and thereafter, all animals were given prophylactic 2 mg/kg flunixin prior to IVT injection, 0.05 mg/kg buprenorphine immediate after IVT injection, and 2 mg/kg flunixin and 0.05 mg/kg buprenorphine at 6 hours after IVT injection. With this regimen, occasionally, only a few animals required 0.05 mg/kg buprenorphine treatment on the morning after IVT injection. From this experience, it appeared that at least for the first 24 hours after IVT injection, there was some degree of ocular discomfort, which was associated with IVT and required intervention and management.

PS 547 DIURON-INDUCED DECREASE OF CD4+ LYMPHOCYTES IN THE SPLEEN OF MALE WISTAR RATS.

S. M. Ihlaseh, A. Domingues, A. F. Cardoso, A. T. Spinardi-Barbisan, M. C. de Oliveira and J. V. de Camargo. *Pathology, Sao Paulo State University - UNESP - Medical School, Botucatu, Brazil.*

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea), an urea-derived herbicide, was shown to induce methaemoglobinemia followed by splenomegaly, hemolytic anemia and compensatory hematopoiesis in rats. The present study, approved by the local Committee for Ethics in Animal Experimentation, evaluated the effects of diuron on the spleen. Six-week old male Wistar rats were exposed through diet to diuron at 60, 125, 500, 1250 or 2500 ppm for different periods up to 20 weeks. Rats fed the two higher levels of diuron for 28 and 90 days were also evaluated by flow cytometry (FC) for the effects on the number of CD4+ T and CD8+ T lymphocytes in the spleen. After 28 days, alterations in the spleen included increased relative weights (+31% and +92% for rats fed 1250 and 2500 ppm, respectively), increased extramedullary hematopoiesis, and depletion of white pulp, particularly the periarteriolar lymphocyte sheaths (PALS). Since PALS is mainly populated by CD4+ T lymphocytes, this observation was consistent with the decreased population of CD4+ T, but not of CD8+ T, as found by FC analysis. These alterations were less significant at the 90th day of exposure and reverted 30 days after diuron withdrawal. After 20 weeks of exposure, increased absolute spleen weights (splenomegaly) was observed in rats fed 1250 (+34%) and 2500 ppm (+46%), associated with a dose-response increased incidence and severity of red pulp congestion. No other relevant histological alteration was registered, besides increased deposition of hemossiderin in larger spleens. Depletion of splenic white pulp was present in animals fed 1250 and 2500 ppm. Due to the lack of effect on CD8+ T lymphocytes, these data suggest that besides the effects of metaemoglobinemia, spleen alterations due to diuron may also result from direct toxicity on CD4+ T cells. Support FAPESP Proc. 06/60506-1 and 08/55644-1.

PS 548 INCREASED SECONDARY BILE ACIDS IN OATP1A1-NULL MICE CAN BE EXPLAINED BY AN OVERGROWTH OF INTESTINAL BACTERIA.

P. B. Limaye[1], Y. Zhang[1], I. L. Csanaky[1], L. Lehman-McKeeman[2] and C. D. Klaassen[1]. [1]*Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS and* [2]*Discovery Toxicology, Bristol-Myers Squibb Co., Princeton, NJ.*

Mouse organic anion transporting polypeptide 1a1 (Oatp1a1, Slco1a1, Oatp1) is predominantly expressed in liver. The role of Oatp1a1 in bile acid (BA) transport was determined by generating Oatp1a1-null mice. Oatp1a1-null mice exhibited higher serum deoxycholic acid (DCA), suggesting a role of Oatp1a1 in DCA uptake. However, plasma clearance of DCA after the intravenous injection was not different between Oatp1a1-null and WT mice, indicating that Oatp1a1 does not play a major role in the hepatic uptake of DCA. To determine whether the higher serum DCA concentration could be due to increased conversion of the primary BA, cholic acid (CA), to the secondary BA, DCA, in the intestine, intestinal BAs were quantified in Oatp1a1-null and WT mice. Secondary BAs, including DCA and lithocholic acid (LCA), were higher in the small (SI) and large intestine (LI) of Oatp1a1-null mice. Oatp1a1-null mice also had increased unconjugated BAs in the SI. It is known that BAs are metabolized to secondary BAs by intestinal bacteria. Therefore, we hypothesized that the increase in secondary BAs in the Oatp1a1-null mice is due to change in the intestinal bacterial profile. The intestinal bacterial population was quantified by developing a novel 16S-rRNA probe-based branched DNA assay. In WT mice, the LI is highly enriched with bacteroides and firmicutes bacteria, whereas the SI has much less bacteria. In contrast, the Oatp1a1-null mice had more than a 1000-fold overgrowth of bacteria in the SI. In the LI of the Oatp1a1-null mice, a striking increase in Bacteroides but a decrease in Firmicutes bacteria was observed. These findings indicate that the increased intestinal bacteria in the Oatp1a1-null mice are responsible for the increased DCA in the blood, as a result of increased conversion of CA to DCA. (Supported by NIH grants ES-09649, ES-09716, DK-081461, and RR-021940 to CDK)

PS 549 TREATMENT PARADIGM FOR ASSESSMENT OF ETIOLOGIES OF PAFURAMDINE-INDUCED KIDNEY AND LIVER TOXICITY.

K. D. DeSmet[1], A. Harrill[1], J. Hall[2], M. Paine[3], M. Wang[3], R. Tidwell[2] and P. Watkins[1]. [1]*Institute for Drug Safety Sciences, The Hamner Institutes for Health Sciences, Research Triangle Park, NC,* [2]*Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC and* [3]*Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC.*

Preclinical toxicity testing commonly involves the use of a single outbred or inbred line of mouse. These models are useful for efficacy studies but may not represent the diversity of adverse responses to a drug present in a genetically diverse human population. Pafuramidine, an oral diamidine prodrug to treat African sleeping sickness, is a prime example of a drug that demonstrated reversible hepatotoxicity, a known effect of diamidines, and no renal toxicity in traditional preclinical testing. Delayed kidney toxicity was not observed until an expanded Phase I trial was undertaken in South Africa. In order to elucidate mechanisms of renal and hepatic toxicity observed for pafuramidine 35 inbred mouse strains will be used to mimic genetic variation that exists in patient populations. A dose-range finding pilot study

has been completed. Male and female CD-1 mice were administered pafuramidine (75 or 125 μmol/kg, i.g.) or vehicle once daily for 2, 4, or 14 days. Animals were euthanized 24 h post-treatment. Left kidney and left liver lobe sections were stained with H&E for histologic analysis. Terminal serum was analyzed for BUN, creatinine, and ALT. Severity of pafuramidine-related pathology changes in both kidney and liver increased in a dose- and duration-dependent manner, with the most severe lesions noted in female mice treated with 125 μmol/kg pafuramidine for 14 days. ALT increased in a dose-dependent manner, demonstrating a clear pafuramidine-induced liver toxicity phenotype. The ability to evoke variable kidney toxicity in nonisogenic CD-1 mice adds supporting evidence to underlying genetic factors that modulate susceptibility to pafuramidine. For the first time, pafuramidine-related renal pathology changes were observed, supporting the mouse as an appropriate model to study diamidine-induced renal and liver toxicity.

PS 550 CO-EXPOSURE OF A NOVEL MOUSE MODEL TO LOW-DOSE TRICHLOROETHYLENE AND INORGANIC ARSENIC: INTERACTIONS, GENETICS, AND RENAL TOXICITY.

M. C. DeSimone[1, 2], G. Glavich[2], A. Bohn[2] and D. Threadgill[1, 2]. [1]*Curriculum in Toxicology, University of North Carolina Chapel Hill, Chapel Hill, NC and* [2]*Department of Genetics, North Carolina State University, Raleigh, NC.*

Co-exposure to low levels of trichloroethylene (TCE) and inorganic arsenic (iAs) through groundwater and dietary contamination occurs in many localities and is thought to result in multiple organ toxicities in susceptible human populations. The interpretation of disease associations with TCE and iAs is under great debate in part due to the lack of an appropriate population-level experimental model with which to test the affect of inter-individual variation in metabolism, detoxification, and transport. To model the genetic heterogeneity of exposed human populations, we developed a unique intercross population derived from FVB/N-*Mdrb1a/1b-/-*, a multi-drug resistant p-glycoprotein knockout mouse model, and CAST/EiJ a wild-derived strain that is genetically distinct from the FVB/N background. By modulating drug transport and genetic variation in the mouse to model human biology, including using a western diet, environmentally-relevant doses of TCE and iAs can be evaluated for their effects on toxicity susceptibility. A study cohort of 900 FVB/N-*Mdrb1a/1b-/-* x CAST/EiJ F3 mice was divided into nine dose groups, each containing 50 female and 50 males, and was administered TCE and sodium arsenite via the drinking water and chow, respectively, at environmentally-relevant concentrations for 52 weeks using a dose-ratio approach. We collected serum and urine in metabolic cages at weeks 0, 16, 32 for evaluation of biomarkers of kidney and other organ injury. Levels of classical biochemical markers serum alanine aminotransferase and blood urea nitrogen showed no significant differences between dose and treatment groups at 16 weeks. However, urinary neutrophil gelatinase-associated lipocalin-2, a sensitive biomarker of early renal injury, was increased 3- to 7-fold over controls at week 16 in mice treated with both TCE and iAs, whereas single treatments alone had no effect. This data suggests that TCE and iAs may cooperate to produce renal toxicity that may be modulated by genetic determinants.

PS 551 LIPIDOMIC CHANGES IN THE LIVERS OF HEPATIC ALCOHOL DEHYDROGENASE-DEFICIENT DEER MICE AFTER SUBCHRONIC EXPOSURE TO ETHANOL.

H. Fernando, K. K. Bhopale, G. A. Ansari and B. S. Kaphalia. *Department of Pathology, University of Texas Medical Branch, Galveston, TX.*

Alcoholic liver disease (ALD) is a 2nd major cause of liver disease resulting in significant morbidity and mortality. ALD begins from early fatty changes and progresses to inflammation followed by fibrosis and cirrhosis. However, the mechanism of ALD is not well understood. In previous studies, we found a significant fatty accumulation (steatosis) and injury in the livers of hepatic alcohol dehydrogenase (ADH)-deficient (ADH-) vs. hepatic ADH-normal (ADH+) deer mice fed ethanol. Therefore, we evaluated dose-dependent lipidomic changes in the livers of ADH- and ADH+ deer mice fed 1, 2 and 3.5% ethanol daily for 2 months. Liver homogenates were extracted with methyl-tert-butyl ether and total lipids and non-esterified fatty acids (NEFA) measured, and lipidomic changes determined using 800 MHz proton nuclear magnetic resonance (1H-NMR) spectroscopy. We found significant increases in total lipids and NEFA only in the livers of ADH- deer mice fed 3.5% ethanol as compared to pair-fed control. Cluster analysis and principal component analysis of 1H-NMR data of liver lipids from ADH- deer mice fed 3.5% ethanol showed increases in total cholesterol, esterified cholesterol, fatty acid methyl esters (FAMEs), triglycerides and unsaturation, and decreases in free cholesterol, phospholipids and allylic and diallylic protons. However, some minor increases were also observed in the livers of ADH+ deer mice fed 3.5% ethanol. Comparison of 1H-NMR data showed increases in total cholesterol, esterified cholesterol, triglycerides, free fatty acids, and decreases in phospatidylcholine, FAMEs, allylic and diallylic protons in ADH- vs. ADH+ deer mice fed 3.5% ethanol. These findings suggest that metabolism of ethanol under hepatic ADH deficiency (a metabolic condition found during chronic alcohol abuse) plays an important role in the formation of fatty liver and its progression to ALD.

PS 552 DEVELOPMENT AND EVALUATION OF A MUCOCILIARY CLEARANCE MODEL IN BEAGLE DOGS *VIA* PLANAR GAMMA CAMERA IMAGE ANALYSIS.

P. Kuehl, R. Chand, M. Doyle-Eisele and J. McDonald. *LRRI, Albuquerque, NM.*

Mucociliary clearance is an important mechanism for clearance of particles from the reparatory system. The quantification of mucociliary clearance has continued to evolve as imaging technologies have evolved. We have developed a beagle dog model for quantification of mucociliary clearance via quantitative analysis of serial planar gamma camera images. In this model beagle dogs (n = 6 for each group) are anesthetized with intravenous propofol throughout the exposures and imaging. The model uses a Harvard pump system to control the respiratory function of the dogs (each with an endotracheal tube in place) during both the treatment aerosol (administered first) and the aerosol delivery of the radiolabeled tracer (administered immediately after the treatment aerosol). The clearance of the technetium-99m sulfur colloid labeled particles was measured via serial planar gamma camera images, acquired with a Siemens SPECT E.Cam. Image analysis was conducted with the Siemens ICON software with regions of interest being drawn around the complete lung. The activity in the lung ROI was then plotted as a function of time and averaged for a treatment group. The slope of the linear relationship is then determined for each group. Statistical comparison of the slopes was performed with a repeated measures analysis of variance model. The utility of this model was evaluated with aerosol delivery of normal saline (0.9%) or 7% saline (hypertonic saline) for 15 minutes. The slopes of each of these were compared to baseline (no treatment). The baseline, normal saline and hypertonic saline slopes were -0.217, -0.230 and -0.272. The hypertonic saline, administered in a manner to mimic clinical administration, was shown to result in a significant increase in mucociliary clearance.

PS 553 REPRESSION OF MULTIPLE CYP2B MEMBERS BY RNAI IN MICE.

B. Damiri, X. Yu, E. Holle and W. S. Baldwin. *Biology/ Clemson University, Environmental Toxicology, Clemson, SC.*

There are few in vivo knockout models available to study the function of Cyp2 members involved in the metabolism of drugs and environmental toxicants. The aim of this work is to produce a potent Cyp2b-knockdown (KD) mouse for subsequent study of Cyp2b function. We made a quintuple Cyp2b-KD mouse using lentiviral-promoted shRNA homologous to all five Cyp2b subfamily members (Cyp2b10, 2b9, 2b13, 2b19, and 2b23). The Cyp2b-KD mice are viable, fertile, and without marked physiological abnormalities except for a marked increase in liver weight of the Cyp2b-KD mice. Expression of the three hepatic Cyp2b members, 2b9, 2b10, and 2b13, is significantly repressed as demonstrated by QPCR and Western blotting. The CAR activator, TCPOBOP was used to determine if shRNA repression could be outcompeted by Cyp2b10 induction. TCPOBOP-treated WT mice show approximately 4X greater expression of Cyp2b than TCPOBOP-treated Cyp2b-KD mice, demonstrating that Cyp induction cannot outcompete the repressive function of the shRNA. In addition to investigating Cyp2b's role in the metabolism of testosterone and parathion, we also observed increased hepatic abdominal and renal fat, especially in individuals younger than 10-weeks old. This suggests that Cyp2b isoforms have an endogenous functions not related to xenobiotic metabolism; perhaps in dietary fat metabolism. Cyp2bs' role in fat metabolism will be investigated besides their role in the metabolism of testosterone and parathion. In summary, we foresee the Cyp2b-KD mouse model as a novel model that significantly improves our understanding of the importance of Cyp2b in chemical sensitivity and drug metabolism.

PS 554 ENDOTHELIAL CELL-SPECIFIC ARYL HYDROCARBON RECEPTOR KNOCKOUT MICE ARE HYPOTENSIVE AND EXHIBIT DIMINISHED RESPONSES TO ANGIOTENSIN II.

L. N. Agbor, M. T. Walsh and M. K. Walker. *Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.*

The aryl hydrocarbon receptor knockout mice (AHR-/-) are hypotensive, associated with reduced contribution of angiotensin (Ang) II to basal blood pressure. We tested the hypothesis that ec-specific AHR knockout (ecAHR-/-) mice will exhibit

a blood pressure phenotype that resembles AHR-/- mice. We mated mice in which exon 2 of AHR is flanked by loxP sites (AHRfx/fx) to mice expressing Cre-recombinase driven by an ec-specific promoter (Tie2), to produce Cre+/AHRfx/fx (ecAHR-/-) as conditional knockouts and Cre-/AHRfx/fx (ecAHR+/+) as controls. AHR excision was confirmed by PCR and blood pressure was measured by radiotelemetry. Further, we measured plasma renin activity and Ang converting enzyme activity, urinary osmolality, hematocrit, and blood Na+ and K+ concentrations as indices of renin-angiotensin system (RAS) activity. In addition, we assessed the blood pressure response to an i.p. injection of Ang II (30 μg/kg). We found that ecAHR-/- mice were hypotensive, compared to ecAHR+/+, with a significant reduction in mean blood pressures (ecAHR-/-: 107.4 ± 2.0 mmHg; ecAHR+/+: 116.1 ± 1.4, n=11/genotype; $p<0.05$). However, the decrease in MAP (~10 mmHg) was less than that observed in AHR-/- mice (~20 mmHg). Pulse pressure, heart rate, and all indices of RAS activity were comparable between genotypes. Interestingly, as observed with the AHR-/- mice, ecAHR-/- mice displayed an attenuated pressor response to acute Ang II. At 5 mins after Ang II treatment, the increase in MAP was comparable between genotypes (ecAHR-/-: 30.2 ± 6.2 mmHg; ecAHR+/+: 42.4 ± 12 mmHg). However, at 30 mins, there was a significant reduction in MAP in ecAHR-/- mice compared to the ecAHR+/+ mice (ecAHR-/-: 9.9 ± 1.5 mmHg; ecAHR+/+: 33.2 ± 1.3 mmHg). ecAHR-/- mice exhibited a sustained decline in MAP as depicted by the area under the curve. These data suggest that loss of AHR from ecs significantly attenuates Ang II responses and is a significant contributor to hypotension observed in AHR-/- mice

PS 555 STUDY DESIGN CONSIDERATIONS FOR ACCURATE CARDIAC TROPONIN EVALUATION IN NON-HUMAN PRIMATES AND RATS.

W. J. Reagan, D. Blackwell, L. Correia, K. Alger, C. Kaplan and F. Geoly.
Pfizer, Groton, CT.

Cardiac troponin (cTn) a biomarker of cardiac degeneration/necrosis is being used more commonly in preclinical safety assessment. To acquire quality samples for accurate assessment, the design of the study should be considered, including timing of the blood sample, restraint and anesthetic used for collection. Eight Cynomolgus monkeys were dosed orally with 0.5 % methylcellulose, 0.1% Polysorbate 80. Blood was collected from the femoral vein at 0, 0.5, 1, 2, 4, 6, 24 hours in chair restrained animals. Animals were returned to their cages after the 2, 4, 6 and 24 hour collections. 5/8 animals had detectable cTn responses (0.2-1.0 ng/ml) using the Siemens Immulite assay, 1-6 hours post treatment. It was hypothesized that they had catecholamine-induced myonecrosis. A second study was done in 3 monkeys that had vascular access ports and well conditioned to the chairing process. They received 9.5 mL/kg (0.9% sodium chloride) intravenously on days 1 and 8 and samples for cTn analysis were taken from the vascular ports 0, 2, 6 and 24 hours post dose on days 1 and 8. cTn in these animals were all <0.2 ng/ml. Heart rates in these animals were typically at their highest value predose (121-187 bpm) and either maintained or decreased over time. Sample collection techniques are also important in rats. To show the effect of common anesthetics used for blood collection in rodents, 5 rats were exposed to 70% CO2/30% 02 at each time prior to jugular venipuncture. Blood was collected at 0, 1, 2, 4, 6, 24, 30, 48 hours. cTn elevations, as determined by the Siemens Centaur first generation assay, occurred in rats at 1-6 hours and peaked at 2 hours (0.47-1.82 ng/ml), but were below 0.07 ng/ml at 0, 24, 30 and 48 hours. Five additional rats were exposed to isoflurane and bled at the same time points and no elevations in cTn (<0.07 ng/ml) were identified. Sample acquisition for cTn analysis should take into consideration not only how and when the samples are acquired, but what has been done with the animal at previous time points.

PS 556 EFFECTS OF TRAINING ON BLOOD PRESSURE MEASUREMENT BY HDO IN CYNOMOLGUS MONKEYS.

B. Niggemann, M. Niehoff, J. Luft and G. Weinbauer. *Covance Laboratories GmbH, Muenster, Germany.*

Determination of blood pressure/pulse rate changes in conscious and restrained animals remains a challenge in safety assessment due to the procedure related effects on systolic and diastolic blood pressure values. High definition oscillometry (HDO) provides clear advantages, e.g. accuracy, when compared to other non-invasive blood pressure measurement techniques, yet also requires animal restraint. This work investigates the effects of animal training on the sensitivity of HDO-based detection of blood pressure alterations.
Four groups of 6 males each received vehicle (group 1, 14% Solutol HS, 6% MCT and 80% Aqua conservans) or a single dose of torcetrapib (30 mg/kg/day) on day 1 and 28. At this dose, torcetrapib increases MAP by 30 mmHg within the first 3 hours post dose when measured by telemetry in the conscious animal. HDO was applied at predose and 3 hrs after dosing. During predose, group 1 and 2 had received a single HDO training session, group 3 had three daily sessions and group 4 >three sessions. At every session, five measurements were performed for each animal. Intra and inter animal coefficient of variation (CV) were lowest in group 4, ranging from 1 to 5% for the systolic pressure (intra animal variation) and 12% (inter animal variation). For group 1, intra animal variation was 2-12 %, with an inter animal variation of 15%, for group 2 the intra animal variation was 1 to 19% and 12% for the inter animal variation, for group 3 it ranged from 2 to 7% and 21% respectively. Group mean changes in the systolic and diastolic pressure between predose and 3 hrs postdose were -34 mmHg in group 1, - 17 mmHg in group 2, - 34 mmHg in group 3 and + 5 mmHg in group 4. Hence, irrespective of the training status and in spite of comparatively lower systolic and diastolic blood pressure variations in trained groups, the established effect of torcetrapib could not be demonstrated using the HDO technique. In conclusion, assessment of cardiovascular effects in restrained conscious cynomolgus monkeys appears less sensitive compared to unrestrained animals.

PS 557 RELIABLE DETECTION OF HYPERTENSION AND HYPOTENSION IN CONSCIOUS, FREELY MOVING CYNOMOLGUS MONKEYS (*MACACA FASCICULARIS*).

M. Niehoff, B. Niggemann, J. Sternberg, U. Zuehlke and G. Weinbauer.
Covance Laboratories GmbH, Muenster, Germany.

The measurement of cardiovascular endpoints in regular toxicology studies remains a challenge in toxicology since currently available methods require restraint animals. In our study we evaluated the recently developed external telemetry system (JET™) for the measurement of hyper- and hypotensive effects in conscious, freely moving animals. The reference compound - Etilefrinhydrochlorid (Effortil®) - directly stimulates the sympathetic nervous system inducing vasoconstriction with corresponding increase in the heart rate, and increased blood pressure. Dihydralazine (Nepresol®) acts as a peripheral vasodilator, thus causing a decrease in the arterial blood pressure. Twelve female cynomolgus monkeys (3.3 to 4.3 kg) were implanted with miniature blood pressure transmitter (PA-C10-TOX-SA) and distributed into 3 groups receiving either vehicle (water, n=4), or 1 mg/kg (n=4) or 10 mg/kg (n=4) of Effortil® on 2 occasions. Following an appropriate wash out phase of 7 days, the animals received again either vehicle (water, n=4), or 1 mg/kg (n=4) or 4 (n=4) mg/kg of Nepresol® during 1 occasion at a dose volume of 2 ml/kg. Telemetric data were recorded for 1 hour predose and 22 hours post dose and analysed using Ponemah software (version 4.9). There was a clear increase in the systolic but not diastolic blood pressure on both occasions from 30 to 240 minutes after dosing 10 mg/kg of Effortil. Around Tmax at 60 minutes post dose, the systolic pressure was 115 mmHg (vehicle), 125 mmHg (1 mg/kg) and 145 mmHg (10 mg/kg) at occasion 1 and 118 mmHg (vehicle), 121 mmHg (1 mg/kg) and 138 mmHg (10 mg/kg). The expected decrease in the diastolic pressure was measured from 30 to 240 minutes post dose following oral Nepresol at 1 and 4 mg/kg. The diastolic pressure was 71 mmHg (vehicle), 61 mmHg (1 mg/kg) and 60 mmHg (4 mg/kg) at Tmax around 45 minutes post dose. The data suggests that the miniature BP transmitter, in conjunction with the JET system is a valid tool to monitor hyper- and hypotension in conscious, freely moving cynomolgus monkeys.

PS 558 STATISTICAL ANALYSIS OF ELECTROCARDIOGRAPHIC (ECG) DATA COLLECTED BY JACKETED EXTERNAL TELEMETRY (JET) AND IMPLANTED TELEMETRY (IMPTLM) IN NONHUMAN PRIMATES.

J. J. Kremer, M. A. Osinski, Z. Xiang, E. Lemke, T. W. Beck and C. M. Foley.
Covance Laboratories Inc., Madison, WI.

Novel study designs are combining toxicology and cardiac safety endpoints that may include analysis of ECG data collected using multiple technologies. A confounding effect for comparing ECG data across technologies is the technical differences, including signal filtering, used by each approach. This study explored whether quantitative ECG data would be comparable across methods when collected simultaneously using JET and ImpTLM. Methods: Four male cynomolgus monkeys were instrumented with an ImpTLM device and outfitted with JET (both from DSI). Data were collected for 20+ hours in a controlled environment and reported as 1-minute averages. Data were filtered for excessive noise using Ponemah. Quantitative ECG values were generated using ECG PRO and analyzed by light or dark phase. Descriptive statistics, Bland-Altman, and regression analyses were used to compare data between JET and ImpTLM. Results: Both methods had a large number of analyzable waveforms in both the light (>1500, JET; >2500, ImpTLM) and dark phase (>23000; >19000). Changes in ECG waveform morphology due to filtering effects were noted and resulted in maximal differences in ECG values between JET and ImpTLM of PR (7 msec), QT (26), and QTc (39) intervals and

QRS (5) duration. However, variability (calculated as %relative standard deviation) over the entire collection was comparable for PR (3.3%, JET; 2.6%, ImpTLM), QT (5.7%, 6.1%), QTc (3.5%, 3.0%), and QRS (2.0%, 1.9%). Bland-Altman and regression analyses showed no consistent bias for data collected using JET or ImpTLM. Discussion: Data collected using JET or ImpTLM was of sufficient quantity and quality for analysis. While the absolute quantitative values varied minimally, both methods had near-equal variances. Taken together, these data suggest that both JET and ImpTLM have a similar ability to detect potential changes in quantitative ECG data and statistical sensitivity when compared in identical study designs and environments.

PS 559 MINIMALLY-INVASIVE TELEMETRIC MEASUREMENT OF ARTERIAL BLOOD PRESSURE IN THE FREELY MOVING MINIPIG. ISOPRENALINE VALIDATION.

S. Baudet[1], S. Milano[1], O. Boucheix[1], E. Chalencon[1], P. Lege[1], C. Bory[1], C. Dupuis[1], E. Rieux[2] and S. Tiesma[2]. [1]*Ricerca Biosciences SAS, Lyon, France and* [2]*Data Sciences International, St. Paul, MN.*

Methods for arterial blood pressure (ABP) measurement by non invasive, Bluetooth-based telemetry in toxicology studies are not as well advanced in comparison to ECG capture. Additional difficulties arise when automated tail cuff measurements of ABP are foreseen in the Göttigen minipig, a species with unsuitable tail conformation and sensitive to stress generated by cuff inflation. We have thus assessed whether ABP signals could be collected in freely moving minipigs by ambulatory telemetry. In particular, we have focused on limiting surgical invasiveness by selecting more readily accessible arteries, by selecting two sizes of telemetry implant device and by assessing signal quality. Isoprenaline (ISO) was subcutaneously administered at the dose level of 30 μg/kg to induce acute hemodynamic changes. The DSI telemetry implants PA-C10-TOX-LA ("C10-Tox") and PA-C40 ("C40") were used. In one animal, the catheter from a C40 and a C10-Tox implant was inserted respectively in the left sub-clavian (SCL) and femoral (FEM) artery. In a second animal, each pressure catheter from two C40 implants was placed in the left SCL and a collateral of the FEM artery (FEMcoll). The best quality signal was obtained with the FEM catheter. Baseline fluctuations and spikes were observed in the FEMcoll signal. Finally the SCL signal presented a more irregular pattern possibly related to kinking or pinching of the catheter. ISO induced a prompt and maximal fall in ABP versus control (~20 mmHg) and a concomitant tachycardia (~90 bpm). The amplitude and time-course of the hemodynamic effects were similar with either implant. ABP can be reliably measured in the minipig with minimal surgery with the pressure catheter from the PA C10 TOX-LA implant inserted in the femoral artery. The resources involved by this approach must be balanced with the ability to continuously and reliably record hemodynamic signals in a species with notoriously variable blood pressure parameters.

PS 560 CLINICAL AND HISTOPATHOLOGICAL OUTCOMES FOLLOWING AMBULATORY INTRAVENOUS INFUSION FOR USE IN PRECLINICAL STUDIES IN THE BEAGLE DOG.

J. Briffaux, C. Gerhardy, M. Chopinaud and N. Pickersgill. *Ricerca Biosciences SAS, Lyon, France.*

A feasibility study was performed to assess the suitability of an ambulatory method of intravenous infusion for use in preclinical studies in the dog and to provide background histopathology data. Five male and 5 female beagle dogs were infused for 2 hours per day with sterile physiological saline at a rate of 10 mL/kg/day for one (4 animals) or four weeks (6 animals). A polyurethane catheter was surgically implanted into the jugular vein with the tip protruding into the anterior vena cava. The distal portion of the catheter was tunneled subcutaneously to exit at the nape of the neck and was connected to a peristaltic pump (Pegasus Light, LAB). The total weight of the pump was approximately 200 g. The pump and 150-mL plastic pouch filled with saline were protected by a polystyrene case contained within a jacket worn by the dog. Standard observations and measurements were performed as in a routine toxicology study including tissue sampling of selected regions of the catheter implantation and injection sites, as well as lungs, liver, spleen, heart and kidneys. The dogs were allowed to socialize in groups of the same sex for 2 hours per day (except during weekends and public holidays), under constant supervision by a technician. There were no adverse findings on ophthalmology electrocardiography, hematology and serum clinical chemistry parameters. The histopathological lesions were considered to be those normally encountered with this route of administration and comparable with in house data generated from the tethered dog model. This experiment demonstrated the feasibility and advantages in terms of socialization of the ambulatory method of intravenous infusion for use in safety studies in dogs.

PS 561 HIGH DEFINITION OSCILLOMETRY (HDO) IN MONKEYS: ACCLIMATION REQUIREMENTS AND SENSITIVITY ASSESSMENT OF HDO *VERSUS* TELEMETRY.

A. Mitchell, A. Lee and T. W. Beck. *Safety Pharmacology, Covance Laboratories Inc., Madison, WI.* Sponsor: P. Kruzich.

Monkeys are commonly used in toxicological research to evaluate drug effects on the cardiovascular system. Obtaining meaningful estimates of blood pressure (BP) and pulse rate (RATE) from conscious, manually restrained monkeys remains a challenge. Animals should be acclimated to the measurement procedure to ensure that unperturbed measurements of BP and RATE are recorded. Eight monkeys implanted with telemetry transmitters (DSI) were acclimated to restraint and HDO measurements (at least 5 readings per animal) on 5 consecutive days. Four days after the last acclimation, 4 animals received L-NAME at 10 mg/kg i.v. and 7 days later clonidine at 0.05 mg/kg i.m. to elevate or lower BP, respectively. The 4 control animals received saline at the respective route. At least 5 HDO readings were obtained for each animal before and at 4 intervals after each drug. The precision and reliability of HDO measurements were compared to simultaneously recorded direct blood pressure data from telemetry implants. Systolic, diastolic, mean arterial pressure (MAP), and RATE were determined. Acclimation data for all parameters were comparable for HDO and telemetry and suggest that animals should be acclimated to the procedure on at least two occasions. Animal remained acclimated to the HDO recording procedure for up to 11 days after the last acclimation session. HDO and telemetry were both able to detect drug induced changes in BP and RATE. Compared to predose values, L-NAME increased MAP by 29 (HDO) vs 19 mmHg (telemetry) four hours postdose, while RATE decreased by 98 (HDO) vs 90 beats per min (bpm) (telemetry). Clonidine decreased MAP by 31 (HDO) vs 27 mmHg (telemetry) one hour postdose, and RATE was decreased by 111 (HDO) vs 101 bpm (telemetry). In conclusion, HDO provides an alternative BP and RATE measurement technique in conscious, manually restraint monkeys when invasive techniques are not warranted.

PS 562 TELEMETRY ECG LEAD PLACEMENT IN CYNOMOLGUS MONKEYS (*MACACA FASCICULARIS*): METHOD TO MAXIMIZE SIGNAL QUALITY.

S. Authier[1, 4], M. Stonerook[2], S. Fournier[1], B. Moon[3] and E. Troncy[3]. [1]*Lab. Research Inc., Laval, QC, Canada,* [2]*Vertex Pharmaceuticals, Cambridge, MA,* [3]*Data Science International, St. Paul, MN and* [4]*Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, QC, Canada.* Sponsor: K. Draper.

Electrocardiograms (ECG) in cynomolgus monkeys are required safety pharmacology investigations. Telemetry enables continuous ECG monitoring and is considered the gold standard to meet regulatory requirements for safety assessments in non human primates. In recent years, different ECG leads have been used with mixed results: Derivation II (DII) subcutaneous ECG leads (significant electromyogram artifacts and low p-wave amplitude); Intracardiac leads (various complications including cardiac chamber perforation); DII ECG leads placement (+) apex of the heart, (-) 4th intercostals space (low P-wave amplitude); Pericardiac ECG lead placement associated with adequate ECG morphology and amplitude with minimal EMG interferences but requires an invasive thoracotomy. The current study evaluated a DII telemetry ECG lead placement with the positive lead sutured to the diaphragm at the level of the heart apex and the negative lead inserted through the jugular vein and advanced to ¾ inch above the right atria as confirmed with fluoroscopy imaging during surgery. This telemetry ECG lead implantation technique resulted in high amplitude QRS complexes (mean R-wave amplitude of 3.5 mV) and P-waves (mean P-wave amplitude of 0.8 mV) which facilitated computerized interval measurements. Average signal to noise ratio was 279.5 for R-wave and 64.0 for P-wave. The technique also provided stable ECG complex morphology with minimally invasive surgical procedures.

PS 563 CHARACTERIZATION OF WELDING AEROSOLS GENERATED BY RESISTANCE SPOT WELDING.

A. Afshari, B. T. Chen, D. Schwegler-Berry, J. Cumpston, A. Cumpston, D. Leonard, S. Friend, P. C. Zeidler-Erdely, D. G. Frazer and J. M. Antonini. *NIOSH, Morgantown, WV.*

Resistance spot welding (RSW) is effective for fabricating sheet metal articles when high rates of production are necessary. RSW is commonly used in the automotive, aircraft, and appliance industries where high speed, repetitive welding is needed and relatively thin section sizes are welded. Decreased lung function, metal fume fever, and chronic bronchitis have been observed after exposure to the complex fumes produced by RSW. The goals of the study were to develop a RSW generation

and inhalation exposure system that will be used for future animal toxicology investigations and to characterize the aerosols and vapors formed during RSW. The system is divided into three different areas: (1) enclosed spot welder, (2) animal exposure chamber including aerosol/vapor characterization equipment, and (3) computer control room. RSW was performed inside of an enclosure as two strips of low carbon steel (1/32 in. thick, 1 in. wide) were continually fed between two copper alloy electrodes and spot welded every 45 sec at a setting of 760 lbs force and 5000 amps. A real-time aerosol monitor (Data RAM) was used to monitor and maintain a particle mass concentration at 1.4 mg/m^3. Particle size distribution and mass median aerodynamic diameter (MMAD) were determined using a Micro-Orifice Uniform Deposit Impactor (MOUDI). Particle morphology and elemental composition were determined by scanning electron microscopy and energy dispersive X-ray analysis (SEM/EDX). Analysis of the size distribution indicated the MMAD of the generated particles was approximately 0.94 μm with a geometric standard deviation of 2.0. SEM/EDX revealed the RSW aerosol particles to be primarily composed of iron and arranged as chain-like agglomerates that resembled the morphology of typical arc welding fume. Larger, more amorphous particles also were observed among the agglomerates. With the development of this novel RSW system, the potential toxic lung effects of spot welding fume can be examined in an animal model.

PS 564 ACUTE INHALATION STUDY OF ALLYL ALCOHOL FOR DERIVATION OF ACUTE EXPOSURE GUIDELINE LEVELS (AEGL).

A. Li[1], J. Fowles[2], M. Banton[2], C. Picut[3] and D. Kirkpatrick[3]. [1]*Exponent Inc., San Francisco, CA,* [2]*LyondellBasell, Rotterdam, Netherlands and* [3]*WIL Research Laboratories, LLC, Ashland, OH.*

An acute whole-body inhalation study for allyl alcohol in Crl:CD(SD) rats was designed to support derivation of AEGL values, with emphasis on establishing NOAEL's for irreversible effects of different exposure concentrations(C)and durations(T). This study also illustrates a practical approach to enhancing the design of acute studies for improved derivation of AEGL values. Groups of 10 rats (5 per sex) were exposed for 1 hr (0, 50, 200 or 400 ppm), 4 hrs (0, 20, 50 or 100 ppm) or 8 hrs (0, 10, 20 or 50 ppm). Clinical examinations were performed by one technician 30 min, 1 and 4 hr into exposure (depending on duration of exposure),and near the end of exposure (including ranked response to a novel noise stimulus). Clinical observations in an open field were made on all animals in random order within 22-71 minutes after exposure was terminated. Clinical pathology, gross necropsy, and histopathology (nasal tissues-6 levels, larynx, trachea, lungs/bronchi, liver, kidneys) were evaluated 14 days after exposure. Mortality was limited to 1 male exposed for 8 hrs to 50 ppm. Clinical findings of gasping, rales, increased respiration, or flushing noted during or 1 hr after exposure were rapidly reversed for all groups. Histopathology in the nasal cavity, which is considered reversible, was noted at all exposure levels following 1,4 or 8 hrs of exposure, with increased incidence at mid and high-dose levels. Severe, irreversible lesions were only present in 50 ppm/8-hr males. No treatment-related findings were observed in the liver and kidneys, or in the lungs of surviving animals. The NOAEL for reversible effects was <50, <20 and <10 ppm for 1, 4 and 8 hrs, respectively. The NOAEL for irreversible effects was >400, >100 and 20 ppm for 1, 4 and 8 hrs, respectively. In comparing treatment levels with same C X T products, the incidence and/or severity of olfactory epithelial degeneration appeared to be positively dependent on the exposure duration.

PS 565 COMPARING ELECTROSTATIC AEROSOL *IN VITRO* EXPOSURE SYSTEM (EAVES) TO TRADITIONAL PARTICULATE MATTER EXPOSURE TECHNIQUE.

K. Lichtveld[1], S. Ebersviller[1], K. G. Sexton[1], H. Jeffries[1] and I. Jaspers[2]. [1]*Environmental Sciences & Engineering, University of North Carolina, Chapel Hill, NC and* [2]*CEMALB, Chapel Hill, NC.*

The majority of *in vitro* studies examining the effects of particulate matter (PM) use techniques involving ambient PM collected on filters, which are subsequently resuspended in a liquid medium and then added to the cell culture. Major shortcomings of such filter collection methods include the potential loss of volatile organic compounds from the PM, agglomeration of particles during collection, and the possible alteration of the particles during the recovery process and while in the liquid medium. To overcome this issue, the Electrostatic Aerosol *in vitro* Exposure System (EAVES), was developed to expose human lung cells directly to particles without prior collection in media. The goal of the study described here is to compare published methods using resuspension of particles collected on filters (~80 μg per cm2) to exposures using the EAVES device (~3.0 μg per insert). Diesel exhaust (DE) was used to determine the efficiency and sensitivity of the respective methods. The DE PM used during these experiments was collected with an outdoor smog chamber capable of mimicking the ambient atmosphere. All emissions were injected directly from either a 1980 Mercedes Benz or a 2006 Volkswagen Beetle and combined with a background of synthetic urban atmosphere. Human respiratory epithelial cells (A549) were exposed to PM in EAVES and filters were collected simultaneously for later resuspended exposures. Following exposures, the toxicological response from the two methods were compared by analyzing IL-8 cytokine production. The data suggest that exposing cells to resuspended PM requires 20x the amount of PM material used in the EAVES device to get similar IL-8 responses. Taken together these results further support the use of the EAVES device and direct exposure of target cells to PM, as it provides an efficient and effective alternative to the more conventional particle *in vitro* exposure methods.

PS 566 VITAMIN C YELLOWING ASSAY STUDIES WITH TITANIUM DIOXIDE AND NONFIBROUS POTASSIUM TITANATE SAMPLES.

D. B. Warheit[1], K. L. Reed[1] and S. Sakai[2]. [1]*DuPont Haskell Lab., Wilmington, DE and* [2]*Otsuka Chemical Company, Osaka, Japan.*

Using animal alternative methodologies for screening lung toxicity of particles is a significant goal. Previously, we assessed the role that particle physicochemical characteristics might play in producing lung toxicity in vivo. Groups of rats were intratracheally exposed to different compositions of nanoscale and fine TiO2 particulates and conventional lung biomarkers were measured over a 3-month postexposure period. It was concluded that particle surface reactivity indices of the TiO2 particulates correlated better with lung toxicity endpoints when compared to other factors such as particle size or surface area measurements. In that study, particle surface reactivity endpoints were measured using the Vitamin C yellowing assay as an indicator of chemical reactivity. Subsequently, it has been proposed that this screening in silico tool might be useful for predicting in vivo pulmonary hazard effects of other titanium containing compounds, such as nonfibrous potassium titanate particulates. Accordingly, the surface reactivity of four different potassium titanate test particulate samples were measured using the Vitamin C yellowing assay; and were compared to previously assessed in vivo pulmonary toxicity effects following short or long-term pulmonary exposures to these test substances in rats. Using Vitamin C yellowing assay methodology, the results demonstrated moderate chemical reactivity for the Terracess TF-S and JS compounds (delta-b values of 8.9 and 9.0, respectively vs. a positive control ultrafine TiO2 value of 28.2), and low delta-b activity for the Terracess LS (0.55) and PS (1.1) particle-types. The finding of moderate delta-b activity for the Terracess TF-S and JS particle samples did not correlate with the finding of very low in vivo pulmonary activity for these particle-types in the lungs of rats. Based upon these results, it seems reasonable to conclude that the Vitamin C yellowing assay may not be an appropriate in silico screening tool for predicting the effects of potassium titanate compounds following pulmonary exposures in rats.

PS 567 SUSTAINED TRANSCRIPTIONAL RESPONSES ACCOMPANY PULMONARY ACROLEIN EXPOSURE IN MICE.

P. C. Burcham, B. Lauder, A. Raso and P. Henry. *Pharmacology Unit, University of Western Australia, Nedlands, WA, Australia.*

P. Burcham, B. Lauder, A. Raso and P. Henry. Pharmacology Unit, University of Western Australia, Perth, Australia.
Acrolein is a noxious 3-carbon electrophile that readily attacks cell macromolecules in exposed tissues. Its presence in smoke produced during the combustion of diverse organic matter ensures acrolein plays an important pathogenetic role in the life-threatening lung oedema that afflicts victims of smoke inhalation injury (SII). Since the transcriptional responses that accompany pulmonary exposure to odedatomatogenic doses of acrolein are poorly characterized, we used Agilent microarrays to monitor time-dependent changes in the levels of 15,000 gene transcripts in mouse lung at various times (0, 2, 4, 8 and 24 h) after the administration of a pneumotoxic dose of acrolein (12 mg/kg). Acrolein was administered via oropharyngeal instillation in a small volume of isotonic saline. Acrolein exposure significantly increased lung wet/dry ratios and protein content in bronchoalveolar fluid after 24 h. RNA was extracted from the lungs of 3 animals per time point. Microarray analysis revealed maximum gene dysregulation occurred 2 h after acrolein dosing, with 565 transcripts altered more than 2-fold at this time relative to controls (315 upregulated and 250 downregulated). Gene upregulation also dominated transcriptional responses at the subsequent time points (71% of 242 responsive genes at 4 h, 62% of 357 altered genes at 8 h, and 67% of 249 responsive genes at 24 h). The transcriptional response to acrolein was very stable, with a

group of 222 genes altered 2-fold or more at 2, 4 and 8 h. Few "new" genes were altered after 24 h. Functional Gene Ontology analysis of the 222 member geneset revealed clusters of genes in pathways involved in cell death regulation (9 genes), inflammatory responses (9 genes), transition metal ion binding (19 genes), DNA binding (15 genes) and response to external stimuli (12 genes). These findings help clarify the mechanisms underlying pulmonary injury and inflammation in the acrolein-intoxicated lung.

PS 568 GAS-PHASE AND PARTICULATE COMPONENTS OF DIESEL EXHAUST PRODUCE DIFFERENTIAL CARDIOPHYSIOLOGICAL IMPAIRMENTS IN HEALTHY RATS.

C. Gordon[1], Q. Krantz[2], P. J. Rowsey[3], M. C. Schladweiler[2], A. D. Ledbetter[2] and U. P. Kodavanti[2]. *[1]Toxicity Asessment Division, U.S. EPA, Research Triangle Park, NC, [2]Environmental Public Health Divsion, U.S. EPA, Research Triangle Park, NC and [3]University of North Carolina, Chapel Hill, NC.*

We recently showed that inhalation exposure of normotensive Wistar Kyoto (WKY) rats to whole diesel exhaust (DE) elicited changes in cardiac gene expression pattern that broadly mimicked gene expression in non-exposed spontaneously hypertensive rats. We hypothesized that healthy WKY rats would develop hypertension when exposed to whole DE but not gas-phase components which includes carbon monoxide, a known inhibitor of blood pressure. Male WKY rats (12-14 wk old) were implanted with radiotelemetry units (Data Sciences) to monitor blood pressure (BP), heart rate (HR), core temperature (CT), QA interval, and motor activity (MA). Rats were placed unrestrained in chambers and exposed for 5 h/d, 5d/wk for 4 wks to filtered air (CON), gas-phase components of filtered DE (FDE), or whole DE consisting of 1.81 mg/m3 of ultrafine particles plus gas-phase components (N=4 per exposure condition). Telemetry parameters were monitored continuously at 10 min intervals during exposure and between exposures while rats were housed in their home cages. DE and FDE led to mild reductions in BP and HR during the first week of exposure. CT and MA were unaffected. QA interval was elevated in DE but not FDE during all four wks of exposure. Telemetry parameters recovered during the recovery phase each night. However, QA interval showed a tendency to remain elevated during recovery from DE. An increase in QA interval is an indication of reduction in cardiac contractility. The data suggest that particulate but not gas-phase components of DE selectively impairs cardiac contractility in a persistent manner in healthy normotensive rats, while causing acute and transient reduction in blood pressure, possibly mediated by gas phase components (Abstract does not represent U.S. EPA policy).

PS 569 A POSSIBLE MECHANISM ASSOCIATED WITH AMIODARONE-INDUCED PULMONARY TOXICITY.

B. M. ALShammari[1], S. AlBakheet[2] and M. Khalifa[2]. *[1]National Food and Drug Authority, Saudi Food and Drug Authority, Riyadh, Saudi Arabia and [2]Pharmacology, King Saud University, Riyadh, Saudi Arabia.*

Pulmonary toxicity is one of the most serious adverse effects associated with the antiarrhythmic drug amiodarone (AM). This study is an extension of our previous work to resolve the controversy regarding the AM-induced pulmonary toxicity (AIPT). To accomplish this goal, Male Wistar rats (n=10/group) were weighed and given AM in a dose level of 80mg/kg/day/i.p for 7, 14, 21 and 28 in consecutive days. Rats were weighed and sacrificed in day 7, 14, 21 and 28 following AM injection. Bronchoalveolar lavage (BAL) was collected to assess total leukocytic count. Lung samples collected from each group were weighed. Homogenization was done freshly and used for determination of ATP content and hydroxyproline levels. Histopathological changes were also evaluated. The AM-treated animals show a significant decrease in body weight, indicated that the animals have reacted adversely to the AM treatment and the indicators of general lung cell injury, including lung weights and lung/ body weight coefficient, were all increased early after AM dosing and remained elevated at every time examined. In rats treated with AM for two weeks, an increased total leukocytic count is observed in BALF and histopathological examination. Such increase is an informative measure of inflammation in the lung. Treatment for two and three weeks produced a significant depletion in ATP level and the lung hydroxyproline of AM-treated groups was significantly increased by approximately 50%, 61%, 71% and 70.6% respectively. Histopathological diagnosis was mostly granulamatous inflammation and thickened alveolar walls in rats treated for three and four weeks respectively. Collectively, these data suggest that the increased hydroxyproline level may correlate as result with edema early after AM administration followed by the inflammation as a consequence days after and Loss of cellular ATP can be considered as subsequent event in AIPT ending ultimately in sever lung toxicity.

PS 570 VARIABILITY IN ONSET OF ECG CHANGES INDICATIVE OF ISCHEMIA AFTER EXPOSURE TO WHOLE *VS.* FILTERED DIESEL EXHAUST IN HYPERTENSIVE RATS. INSIGHT ON MECHANISM?

C. M. Lamb[1], N. Haykal-Coates[2], A. P. Carll[3], M. S. Hazari[2], D. W. Winsett[2], D. L. Costa[4] and A. K. Farraj[2]. *[1]Toxicology, University of North Carolina-Chapel Hill, Chapel Hill, NC, [2]EPHD, U.S. EPA, Research Triangle Park, NC, [3]EHE, UNC School of Public Health, Chapel Hill, NC and [4]ORD, U.S. EPA, Research Triangle Park, NC .*

Diesel exhaust (DE) is a complex mixture of gases including CO_2, O_2, NO_2, CO, aldehydes, benzene, and polycyclic aromatic hydrocarbons (PAHs) as well as highly respirable particulate matter. DE is a significant component of fine particulate matter ($PM_{2.5}$) air pollution, which itself has been positively associated with hospital admissions and cardiovascular morbidity and mortality, especially in individuals with pre-existing cardiovascular diseases including hypertension. We hypothesized that diesel exhaust exposure will result in concentration dependent cardiac dysfunction in hypertensive but not normal rats. Spontaneously hypertensive (SH) and Wistar Kyoto (WKY; rats with normal blood pressure) rats, were implanted with biopotential radiotelemetry transmitters to monitor electrocardiogram (ECG) and heart rate (HR), and exposed once for 4 hours to 150ug/m^3 or 500ug/m^3 of whole (WDE; gases + PM) or filtered (FDE; gases alone) diesel exhaust, or filtered air (control) in whole body exposure chambers. Only the FDE, but not the WDE, caused decreases in HR during exposure at both concentrations in SH rats. In addition, only the low concentration of FDE caused ST depression (a change in the ECG often associated with myocardial ischemia) during exposure in SH rats, and this change persisted 18 hours after exposure. DE exposure also caused a decrease in HR in normal rats but did not affect ST amplitude. Taken together, the data suggest that hypertension may predispose to the potential ischemic effects of DE and that the components of DE may have divergent effects with some eliciting immediate irritant effects (e.g., gases) while others (e.g., PM) triggering persistent effects potentially via separate mechanisms. (This abstract does not reflect EPA policy).

PS 571 THE ROLE OF RELMα (RESISTIN LIKE MOLECULE α) IN THE RESPONSES OF THE LUNG'S VASCULATURE TO ANTIGEN AND URBAN PARTICULATE MATTER.

G. Grunig, M. Sisco, T. Gordon and C. Hoffman. *Environmental Medicine, New York University, Tuxedo, NY.*

Mouse RELMα is a member of the resistin family of adipokines and is expressed by many cell types including intestinal and airway epithelial cells, macrophages activated in the course of Th2 responses (alternatively activated) and white adipose tissue. This molecule is also known as Found in Inflammatory Zone (FIZZ) and Hypoxia Induced Mitogenic Factor (HIMF). The human homologue, RELMβ, is expressed at increased levels in the pulmonary artery in pulmonary arterial hypertension and is a mitogen for human vascular smooth muscle cells.
Our studies focused on the role of RELMα in severe pulmonary arterial remodeling induced by a T helper 2 (Th2) response to antigen. We have shown that the number of cells that stain positively for RELMα by immunohistochemistry was significantly correlated with the severity of pulmonary arterial remodeling. Furthermore, our studies showed a significant exacerbation of pulmonary arterial remodeling in wild type mice exposed to a combination of antigen and urban particulate matter. RELMα deficient mice, in contrast, failed to show the exacerbation and developed pulmonary arterial thickening to exposure with antigen and respirable urban particulate matter to a significantly smaller degree relative to wild type. The severity of inflammation and the type of the immune response in the antigen challenged RELMα deficient mice was comparable to wild type. Because RELMα is known to have a critical role in hypoxia induced pulmonary arterial remodeling, and hypoxia-mimetic metals have been identified in respirable urban particulate matter, our data suggest that cellular signals induced by the Th2 response synergize with hypoxia-(mimetic) induced signaling via the shared mediator, RELMα. This knowledge is significant for the understanding of the maintenance of the pulmonary arterial bed in the lungs.

PS 572 TOXIC LOAD METHOD FOR CALCULATING INHALATION DOSES FOLLOWING TOXIC VAPOR EXPOSURES.

S. Chesler[1], H. Salem[1] and J. Moser[2]. *[1]Edgewood Chemical Biological Center, Gunpowder, MD and [2]Chemical Security Analysis Center, Aberdeen Proving Ground, MD.*

The U.S. Army's Edgewood Chemical Biological Center (ECBC), has established procedures and practices for characterizing human exposures to chemical warfare agents and other toxic compounds. In order to effectively calculate dose, morbidity

and lethality associated with these types of exposures, the use of mathematical tools not ordinarily utilized in conventional toxicology are necessary. These tools are based, not on the simplistic Haber's Rule of dose calculation, but on the more rigorous Toxic Load concept of exposure. Haber's Rule: Dose = C x t; Toxic Load: Dose = Cn x t; where C is the concentration of the toxic gas or aerosol, n is the experimentally determined toxic load exponent, and t is the exposure time. Using Haber's Rule, median lethal dose (LCt50) is defined as the product of C and t at which 50% of the exposed population suffers death. This definition assumes that LCt50 is constant as exposures times decrease and concentration increases, but this assumption is usually found to be fallacious for most volatile compound and it is experimentally observed that as concentration increases LCt50 tend to decrease, i.e. as the concentration increases the toxicity appears to increase. This is illustrated in the Table below: Data in the last column assumes that n is equal to 1.5, and since the resulting values in this data set are essentially identical validates that assumption. Actual reported data will be used to illustrate how to calculate the Toxic Load constants. Modeling will be used to calculate the overestimation of casualties that will occur if Haber's Rule is applied when n is not equal to one. Models for both outdoor and indoor chemical releases will be shown and compared. Actual reported accidental releases will be used as examples. The thermodynamics of large releases will be discussed to show that simple assumption of flash evaporation leads to very significant errors in dispersion calculations. The effect of experimental errors, both random and systematic, will be determined.

PS 573 PRELIMINARY ASSESSMENT OF PULMONARY TOXICITY OF MIDDLE EASTERN SOIL EXTRACT IN A RAT MODEL.

M. L. Foster[1], K. H. Taylor[1], M. G. Stockelman[2], J. A. Centeno[3] and D. C. Dorman[1]. *[1]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, [2]Naval Medical Research Unit, Dayton, Wright-Patterson AFB, OH and [3]Armed Forces Institute of Pathology, Washington, DC.*

Soldiers deployed to the Middle East are exposed to potentially high concentrations of airborne particulate matter (PM) from local soils. In this study, we characterized the respiratory toxicity of the soluble components of Middle Eastern Dust [MED]. MED was irradiated, mixed with PBS, and incubated overnight. The soluble extracts were screened for cytotoxicity using the MTT metabolism assay in a rat lung epithelial cell culture [RLE-6TN]. The in vivo study examined the effects of MED extract after a single intra-tracheal dose. Adult male CD rats were randomly assigned to PBS Control [CN], Camp Victory [CV], Afghanistan [AF], and Taji [TJ] groups. Rats were anesthetized and dosed with 500 µl of MED extract intratracheally. Rats were killed at 8, 24, 72 h, 2 or 4 wk after dosing. Broncho-alveolar lavage fluid [BALF] was collected from the left lung and total cell count, differential cell count, lactate dehydrogenase [LDH] activity, and total protein concentration were determined. TJ and AF were significantly more cytotoxic in vitro than CV or CN. In the in vivo study, a subset of TJ rats demonstrated acute respiratory distress and 3/40 animals from the TJ group and 1/40 CV died within 30 min of dosing. Body weight gain changes in treated rats compared with CN were not significant. Total BALF cell counts showed significant increases in the AF/8h and TJ/4wk group. BALF LDH activity was significantly increased compared to CN for TJ/72h. BALF protein values were unaffected by MED extract exposure. Metal analysis of the extracts demonstrated different metal profiles that likely contributed to these observed differences in response. Our data indicate that the inflammatory response seen in rats exposed to MED extracts was complex, depending on geographic region of the MED and sampling time. Cytokine, histopathology and tissue metal analyses are underway and may prove helpful in further characterizing the effects of MED extract.

PS 574 THE CIGARETTE ADDITIVE MENTHOL BLOCKS THE RESPIRATORY TRACT IRRITATION RESPONSE TO ACROLEIN, A PRIMARY IRRITANT IN CIGARETTE SMOKE.

D. N. Willis[1], B. Liu[2], J. B. Morris[1] and S. Jordt[2]. *[1]Toxicology Program, University of Connecticut, Storrs, CT and [2]Pharmacology, Yale University Medical School, New Haven, CT.*

Menthol, a compound derived from peppermint, produces a cooling sensation which is thought to be mediated via neuronal TRPM8 channels. Menthol is widely used in preparations for the treatment of cough, pain and itch and is a common additive to cigarettes. The current study was performed to characterize the effects on menthol on the respiratory tract irritation response induced by two irritants in cigarette smoke: acrolein and acetic acid. Acrolein is an electrophilic irritant which acts through the TRPA1 receptor. Acetic acid is an acidic irritant that likely acts through the ASIC receptor. Towards these ends female C57Bl/6J mice were exposed to menthol, irritant, or the combination of the two for 15 minutes during which time respiratory parameters were measured. The primary response to irritant vapors in the mouse is sensory irritation. The response, mediated by the trigeminal nerve, is characterized by reduced breathing frequency due to braking at the onset of each expiration. The average duration of braking (DB) during exposure was used to quantify this response. At a concentration of 16 ppm menthol exerted a minimal transient sensory irritation response (50 msec DB). Acrolein (2 ppm) exerted a strong irritation response (200 msec DB). The response to acrolein was blocked by concurrent exposure to 16 ppm menthol. This level of menthol is significantly lower than that present in smoke from mentholated cigarettes. Menthol partially diminished the irritation response to acetic acid, another irritant present in cigarette smoke. It is not known if the counter-irritating effects of menthol are mediated via interactions of menthol with the TRPA1 and/or ASIC receptor and/or via TRM8-mediated pathways. The inhibition of the irritation response to two irritants present in cigarette smoke suggests that mentholated cigarette smoke may contain pharmacologically active concentrations of menthol vapor.

PS 575 THE USE OF E-CADHERIN IMMUNOFLUORESCENCE IN PULMONARY TOXICOLOGIC PATHOLOGY STUDIES.

L. A. Battelli, V. Castranova, D. W. Porter, S. Friend, D. Schwegler-Berry, P. Willard and A. F. Hubbs. *HELD, NIOSH, Morgantown, WV.*

E-cadherin is a calcium dependent adhesion molecule with important roles in epithelial intercellular adhesion and cellular structure. Immunofluorescent staining can localize and quantify protein expression in cells or tissues. Co-localization of 2 or more different proteins used in combination with fluorochromes of different colors can reveal the location of each protein. We investigated the use of e-cadherin immunofluorescence in different species (rat and mouse), with different fixatives (10% neutral buffered formalin, 4% paraformaldehyde, and 2.5% glutaraldehyde), with and without antigen retrieval, and as a dual label with immunofluorescence for other proteins. Mouse monoclonal anti-e-cadherin antibodies (BD Biosciences, San Jose, CA) in conjunction with fluorochrome-conjugated donkey anti-mouse antibodies clearly delineated sites of e-cadherin expression in lungs of rats or mice. EDTA heat-induced epitope retrieval was required to restore antigenicity to fixed tissues. E-cadherin could be demonstrated in formalin or paraformaldehyde fixed tissues but not in glutaraldehyde fixed tissue. Immunofluorescent double-labeling with e-cadherin can be used with immunofluorescent detection of podoplanin, a lymphatic endothelial cell marker that is also expressed by alveolar type I cells in the lung. Low levels of e-cadherin expression in type I cells allowed the double labeled type I cells to be distinguished from lymphatic endothelium and facilitated diagnosis of lymphangiectasia. E-cadherin immunofluorescent double labeling can also be used with activated caspase 3 or β-catenin immunofluorescence to localize the expression of these proteins in damaged airways. Thus, in the lung, e-cadherin immunofluorescence can demonstrate abnormal and normal epithelial cell junctions, facilitate demonstration of airway epithelial changes, and distinguish podoplanin-expressing alveolar type I cells from lymphatic endothelium.

PS 576 TELEMETERED THORACIC IMPEDANCE PNEUMOGRAPHY: COMPARISON WITH AMBULATORY RESPIRATION MEASUREMENT STANDARDS IN THE ANESTHETIZED BEAGLE DOG AND PHARMACOLOGICAL VALIDATION.

S. Milano[1], C. Bory[1], S. Baudet[1] and B. Moon[2]. *[1]Ricerca Biosciences SAS, Lyon, France and [2]Data Sciences International, St. Paul, MN.*

Recent developments in thoracic-impedance-pneumography (TIP) as a telemetry based approach to measure respiratory activity seems very suitable for assessment of pharmacological effects of drugs in non-restrained conditions, and for combination with ambulatory telemetered cardiovascular investigations in toxicology studies. As a first validation step, we have implemented TIP in the anaesthetized beagle dog and compared the respiratory parameters with existing, non-restrained respiratory assessment methods. Four dogs were implanted with DSI D70-PCTR transmitters whose pressure catheters were placed intrathoracically for pleural pressure (PP) measurement. Four weeks later, the animals were isoflurane-anesthetized and instrumented with a tracheal pneumotachometer (PNT) used as the reference method, and a JET® RIP (Respiratory Inductive Plethysmography, DSI) system. One hour after induction, baseline data was collected for 15 min, followed by experimental modifications of the respiratory function: three hypercapnia levels (5.3, 5.8 and 6.1 % ETCO2, 15 min recovery/level) followed by a subcutaneous bolus of Morphine (M) at 2 mg/kg. Three days later the same procedures were repeated replacing Morphine with Buspirone intramuscularly administered at 3 mg/kg. Regression analysis was conducted for the PNT, RIP, TIP and PP based tidal volume (TV), respiratory rate (RR), and minute ventilation (MV) values. When comparing values between RIP, TIP or PP versus PNT, correlation coefficients (R2)

were respectively: 0.57, 0.95 and 0.83 for VT, 0.96, 0.95 and 0.99 for RR and 0.96, 0.97 and 0.98 for MV. These data obtained in controlled conditions (anesthetized dog) strongly support the use of TIP and PP approaches for assessment of TV. All methods yielded excellent results for the measurement of respiratory rate and minute volume. Ultimately, such results also provide control data for studying the effect of similar pharmacological maneuvers in freely moving animals.

PS 577 INHALATION CAPSULE DOSER – CONSERVING TEST ARTICLE IN SMALL SCALE INHALATION EXPOSURES.

S. A. Moore[1], R. Goodway[1], C. Hardy[1], D. Coombs[1], A. Allen[1], S. Cracknell[2], G. Paul[3], G. Somers[3], A. Smith[3], A. Taylor[3], S. Sparrow[3] and D. Hassall[3]. *[1]Inhalation, Huntingdon Life Sciences, Huntingdon, United Kingdom, [2]Huntingdon Life Sciences, East Millstone, NJ and [3]GlaxoSmithKline (GSK), Ware, United Kingdom.*

GSK and HLS have collaborated to identify methods of aerosol generation that minimise test article usage for small scale studies. Following a review of available powder generators, the HLS capsule device was selected for evaluation. Capsules containing 0.1 or 1.0mg of undiluted test article or 40% lactose powder blend were prepared. The device using blended or undiluted 1mg capsules, gave aerosol concentrations (AC) of 8.08 and 26.0μg/L respectively. Two replicate trials using 0.1mg undiluted test article gave mean AC of 0.698 and 0.628μg/L. AC were consistent (%CV <12%) throughout generation periods. The analytical mass median aerodynamic diameters (MMAD) were between 2.3 and 2.5μm and consistent with commercially available generators. The gravimetric MMAD was 3.1μm for the blended material reflecting the presence of larger lactose particles.
Chemical analysis of 0.1 and 1mg capsules indicated that achieved mean masses of dispensed material were in good agreement with nominal fill weights (<1% from target), albeit more variable for the blended material and 0.1mg capsules (<9%).
The generation efficiencies for the capsule generator were lower (6-20%) than those typically achieved using commercially available powder generators (40%). However, overall compound usage for studies up to 10 days at comparable AC was significantly lower due to the elimination of the generator dead space. For example, a target dose of 0.3mg/kg/day, the capsule device would require only 160mg compared with >1g for either a Rotating Brush or Wright Dust Feed generator.
The capsule doser provides consistent AC and reduced compound usage compared with other current technologies. In addition, it is currently being redesigned to decrease usage still further.

PS 578 *IN VITRO* MACROPHAGE CYTOTOXICITY OF INDIUM-PHOSPHIDE AND INDIUM-TIN OXIDE PARTICLES IS DEPENDENT UPON PHAGOSOME ACIDIFICATION AFTER PARTICLE UPTAKE.

W. M. Gwinn[1], C. J. Shines[1], R. W. Bousquet[2] and D. L. Morgan[1]. *[1]Laboratory of Toxicology and Pharmacology, NIEHS, Research Triangle Park, NC and [2]Alion Science and Technology Corporation, Research Triangle Park, NC.*

Indium phosphide (InP) and indium-tin oxide (ITO) are sintered metal particles used extensively in the microelectronics industry, for example as semiconductors and components of flat panel displays such as LCD screens. Inhalation exposure to InP and ITO have been associated with the development of pulmonary diseases in factory workers. Airway-delivered InP or ITO particles exhibit pulmonary toxicity in mice; however, the mechanism(s) of pathogenesis is unclear. Because InP and ITO particles are initially phagocytosed by alveolar macrophages within the lung, we hypothesized that phagosome acidification after particle uptake by macrophages resulted in solubilization of the sintered InP and ITO metal particles releasing free indium, the cytotoxic entity. In this study, we characterized the in vitro cytotoxicity of InP and ITO particles on macrophages after particle uptake. The mouse macrophage cell line RAW 264.7 was treated with InP or ITO for 1.5 hr to allow particle uptake. The cells were then rinsed with media to remove extracellular particles and cultured for 24 hr. Cytotoxicity was measured after 24 hr using the MTT and LDH assays. InP and ITO-treated RAW 264.7 cells exhibited increased cell death relative to media-treated controls. Similar cytotoxic effects were observed after treatment and uptake of particles by BAL-derived primary mouse alveolar macrophages. Pre-treatment of RAW 264.7 cells for 30 min with 25 nM bafilomycin A1, an inhibitor of phagosome acidification, followed by treatment for 1.5 hr with InP (100 μg/ml) or ITO (300 μg/ml) + 25 nM bafilomycin A1 still resulted in particle uptake, but the cytotoxicity of InP and ITO particles was blocked. These data indicate that phagosome acidification after particle uptake by RAW 264.7 cells in vitro is required for the macrophage cytotoxicity of InP and ITO metal particles. These results also support the hypothesis that particulate indium compounds require solubilization in order to be toxic.

PS 579 THE EFFECT OF PARTICLE SIZE AND SEASON ON THE TOXICITY OF URBAN AND RURAL PM.

J. E. Mirowsky[1], C. Hickey[1], M. Lippmann[1], R. Peltier[2], L. Horton[1], K. Galdanes[1] and T. Gordon[1]. *[1]Environmental Medicine, New York University, Tuxedo Park, NY and [2]Environmental Health, University of Massachusetts, Amherst, MA.*

PM is routinely observed as having significantly different chemical composition and total mass concentration based on location, season, and particle size. Health effects induced by fine and ultrafine PM have been extensively studied, but little research has been focused on coarse PM. An important issue in studying the adverse health effects of ambient PM is its heterogeneity throughout different geographical regions and its variation within each region from urban to rural areas. We hypothesized that differences in the toxicity of coarse PM are due to the source contributions to the particles. Approximately 250 samples were collected from 2 urban and 3 rural sites in the metropolitan NYC area in both the summer and wintertime. Particles were collected with a high volume cascade impactor at 900 L/min on polyurethane and polypropylene substrates and were extracted by sonication in water. A human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) and a bronchial epithelial cell line (BEAS-2B) were exposed to PM at a concentration of 50 μg/mL. No significant cytotoxicity, as measured by LDH release, was measured in either cell line at this dose. Reactive oxygen species (ROS) levels were measured by intracellular oxidation of the fluorescent dye 2',7'-dichlorofluorescein. It was observed that the location, season, and size fraction contributed to differences in ROS formation, with the urban, winter, and coarse fractions dominating. A FVB/N mouse model, investigating inflammation via polymorphonuclear neutrophils (PMNs), showed a similar trend. These results support the notion that location, season, and particle size elicit different toxicities, and chemical composition could play a major role in these differences. Future works aims at correlating these findings with the chemical composition of the particles.

PS 580 BIOMARKERS FOR NASAL RESPONSE TO NAPHTHALENE VAPOR.

J. B. Morris[1], L. S. Van Winkle[2], J. Cichocki[1] and A. R. Buckpitt[2]. *[1]Toxicology Program, University of Connecticut, Storrs, CT and [2]Veterinary Medicine, University of California Davis, Davis, CA.*

Naphthalene (NA) is a nasal carcinogen in the rodent inducing significant increases in nasal olfactory neuroblastomas and nasal respiratory adenomas in rats chronically exposed to 30 ppm NA. The mode of action relative to carcinogenesis is unknown although activation by cytochrome P450 is thought to represent a critical step and regenerative cell proliferation following cytotoxicity is likely of importance. The current study is aimed at examining mechanistically relevant biomarkers for acute response to NA that can be applied to examine the regional (e.g. respiratory versus olfactory mucosa) nasal response to this agent. Towards these ends biomarker levels were examined in animals exposed for 1, 2 or 6 hours to 15 or 30 ppm NA. Oxidized and reduced glutathione (GSSG, GSH) levels were assessed to provide markers of oxidative stress and/or electrophile production in tissues dissected from specific regions of the rat nose. In a novel technique, nasal lavage was performed following NA exposure with fluid containing ethidium homodimer1. Ethidum incorporation into nuclei provides an index of nasal cytotoxicity. Nasal GSH levels were rapidly depleted by NA and reached steady state within 1 hour of exposure. At 30 ppm, nasal respiratory and olfactory mucosal GSH levels were depleted to 40% and 80% of control, respectively. GSSG/GSH ratios were not markedly altered. These results suggest NA is activated to an electrophile in both respiratory and olfactory epithelium, but that marked oxidative stress does not occur. Concentration dependent nuclear ethidium staining was observed in nasal epithelium of rats exposed to 15 or 30 ppm NA indicating a cytotoxic effect of NA. These biomarker methods provide region-specific assessment of nasal responses to NA. Regional tissue dissection and histochemical mapping of ethidium incorporation may provide useful tools to examine site selective responses to this carcinogenic vapor. [Supported by the Naphthalene Research Council, ES04311 and ES04699]

PS 581 AMBIENT PARTICLE COMPOSITION AND MECHANISMS OF ENDOTHELIAL DYSFUNCTION IN A MURINE MODEL.

A. Cuevas, E. Liberda and L. Chen. *Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

Our previous studies have shown that various emission sources, sizes, and composition of PM can induce adverse cellular and cardiovascular responses; the exact roles of each individual component responsible for vascular dysfunction are not well understood. Therefore an exploratory study was conducted to examine the effects of aspirated ambient particles from different locations, each having a unique PM com-

position, on vascular reactivity and on mediators of vascular tone. One group of male FVBN mice received a single aspirated (50µl@1mg/ml) of PM (ultrafine, UF: <0.1µm; fine, FP: 0.10-2.5µm; coarse, CP: 2.5-10µm) from 5 sites within the U.S.- Sterling Forest, NY; Manhattan, NY; Seattle, WA; Ann Arbor, MI; Anaheim, CA (N=6 per site). A separate group received multiple aspiration exposures twice a week for either 1 or 3 weeks of fine PM from 2 U.S. locations; Sterling Forest, NY and Manhattan, NY (N=12 per time point/site), and a saline control (N=4). Bronchoalveolar lavage fluid (BALF) was collected to determine pulmonary inflammation. Secondary order mesenteric arteries were removed, cut into 2-mm segments and were mounted on a tension myograph system to measure contractile response 2 and 24-hrs post-exposure. The analyses of BALF revealed pulmonary inflammation in both single and multiple aspiration groups. Compared to the saline exposed mice, no significant differences in the contractile response to phenylephrine (PE) were observed in single aspirated group. Data from both single and multiple aspirations indicates a decrease in relaxation after administration of acytelcholine (ACh) as compared to the controls. These results suggest that short-term PM exposure can induce source-specific pulmonary inflammation and vascular dysfunction.

PS 582 GENERATION AND CHARACTERIZATION OF A CYP2F2-NULL MOUSE MODEL AND STUDIES ON THE ROLE OF CYP2F2 IN NAPHTHALENE-INDUCED TOXICITY IN THE LUNG AND NASAL MUCOSA.

L. Li[1, 2], Y. Wei[1, 2], L. Van Winkle[3], X. Zhou[1], J. Hu[1], F. Xie[1, 2], K. Kluetzman[1] and X. Ding[1]. *[1]Wadsworth Center, New York State Department of Health, Albany, NY, [2]School of Public Health, State University of New York at Albany, Albany, NY and [3]Center for Health and the Environment, University of California-Davis, Davis, CA.*

Our aim was to generate a Cyp2f2-null mouse model for studies on the toxicological significance of CYP2F2 in vivo. The CYP2F enzymes, abundantly expressed in the respiratory tract, are active toward many xenobiotic compounds, such as naphthalene (NP). However, the precise roles of these enzymes in tissue-selective chemical toxicity have been difficult to resolve. The null mouse was generated, on the C57BL/6 strain, by replacing the Cyp2f2 3rd coding exon with a neo gene. Homozygous Cyp2f2-null mice, which had no CYP2F2 expression, and did not show any changes in the expression of other Cyp genes examined, were viable and fertile, and without any in utero lethality or developmental deficits. The loss of CYP2F2 expression lead to substantial decreases in the in vitro catalytic efficiency of microsomal NP epoxygenases in lung (~70 fold), liver (~4 fold), and olfactory mucosa (~6 fold), and significant decreases in rates of systemic NP (300 mg/kg, i.p.) clearance. The Cyp2f2-null mice were largely resistant to NP-induced cytotoxicity, when examined at 24 h after NP dosing (at 300 mg/kg, i.p.), and to NP-induced depletion of total nonprotein sulfhydryl (NPSH), examined at 2 h after dosing, in the lungs. In contrast, the loss of CYP2F2 expression did not alleviate NP-induced NPSH depletion or tissue toxicity in the olfactory mucosa. Clearly, mouse CYP2F2 plays an essential role in the bioactivation and toxicity of NP in the lung, but not in the olfactory mucosa. The Cyp2f2-null mouse should be valuable for studies on the role of CYP2F2 in the metabolism and toxicity of numerous other xenobiotic compounds, and for future production of a CYP2F1-humanized mouse. (Supported in part by NIH grant CA092596 and ES007462 and a grant from the Styrene Information and Research Center) (The first two authors made equal contribution)

PS 583 GENDER-RELATED DIFFERENCES IN AIR POLLUTION – MEDIATED EXPRESSION OF FACTORS ASSOCIATED WITH PROGRESSION OF ATHEROSCLEROSIS.

A. K. Lund, J. Lucero, L. Blair and J. D. McDonald. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

Epidemiological studies report a causative correlation between traffic-related air pollution and exacerbation of cardiovascular disease; however, the underlying mechanisms are largely unknown. We have previously reported that vascular ROS are elevated in male apolipoprotein KO (ApoE-/-) mice exposed to vehicular emissions, resulting in increased expression of oxidized LDL (oxLDL), the lectin-like-ox-LDL receptor (LOX-1), endothelin-1 (ET-1), and matrix metalloproteinases (MMPs). To determine if there are gender-related differences resulting from exposure to inhaled air pollutants, 10 wk old female ApoE-/- mice, on a 20% fat diet, were assigned to receive either filtered-air [FA: ovariectomized (OV-): n=10, and non-ovariectomized (OV+): n=10] or a mixed exposure of 250µg PM/m3 diesel + 50µg PM/m3 gasoline exhausts (ME: n = 10 OV-; n=10 OV+) for 6 hr/d for 7 d, based on estrus cycle. Analysis of aorta from ME-exposed OV+ females showed elevations in TBARS (16.5 vs. 9.9 nmol/ml/mg), while oxLDL and expression of LOX-1, ET-1, and MMP-9 mRNA were only minimally increased. Interestingly ME-exposure significantly increased CYP1A1 expression (2.9-fold) in OV+ females, which is observed to be decreased in male-exposure studies. Expression of tissue-inhibitor of MMPs (TIMP-1) was increased with ME-exposure in OV+ mice (2.6-fold), while TIMP-2 expression showed no change, both of which are in opposition to that observed in similar male studies. ME-exposure in OV- female mice showed an even further increase in vascular TBARS compared to FA OV- mice (24.3 vs. 8.5 nmol/ml/mg) and a significant elevation in expression of LOX-1 (2.5-fold), ET-1 (2.3-fold), MMP-9 (2-fold), and TIMP-2 (2.1-fold) mRNA expression, while there was no change in CYP1A1 or TIMP-1 expression. Such findings suggest that female hormones likely play a (protective) role in the inhibition of induction of ROS and expression of vascular factors associated with progression of atherosclerosis, in response to inhalational exposure to vehicular emissions. Funded by NIH R00ES016586.

PS 584 CHANGES IN MOUSE PULMONARY RESPONSES AS A FUNCTION OF METHACHOLINE AEROSOL CHARACTERISTICS.

B. A. Haghverdian[1], L. Mendez[2], A. Keebaugh[1], G. Gookin[1] and M. Kleinman[1]. *[1]Medicine, University of California, Irvine, Irvine, CA and [2]Microbiology and Molecular Genetics, University of California, Irvine, Irvine, CA.*

The determination of pulmonary resistance (RL) and dynamic compliance (Cdyn) after a bronchoconstrictor challenge is the gold-standard parameter measured in airway responsiveness studies in animal models of asthma. Methacholine (MCh) is a bronchoconstrictor that provokes lung airway narrowing that is commonly used in airway responsiveness studies to evaluate the toxicity of air pollutants, such as particulate matter. The sensitivity of this assay is influenced by the design of the nebulizer and its output parameters such as the particle size distribution and mass output of the aerosol. Therefore, the goals of this study were to: 1) evaluate the output characteristics of two commercial nebulizers, and 2) determine which nebulizer will elicit the most reproducible bronchoconstriction in the BALB/c mouse. A cascade impactor was employed to determine the gravimetric output and particle size distribution (PSD) of the aerosols. Differences in both gravimetric output and PSD were observed when the nebulizers were operated at the same pressure. MCh aerosols from each nebulizer were then delivered to tracheally intubated BALB/c mice to determine whether physiological pulmonary responses differed based on the intrinsic characteristics of each nebulizer. While there was no observed difference in Cdyn, there was a 1.5 fold increase in RL of mice exposed to MCh aerosols generated with one of the tested nebulizers. This can be explained due to differences in the mass output and PSD of the two nebulizers. Indeed, the predicted deposition of MCh aerosol in the tracheobronchial region of BALB/c mice was estimated to be 2.2 fold greater for the nebulizer that elicited a greater pulmonary function response. These results emphasize the need to properly characterize aerosol generation systems to increase the sensitivity and reproducibility of pulmonary function tests for inhalation toxicology studies.

PS 585 ACUTE INHALATION SAFETY ASSESSMENT OF ACETYLATED LECITHIN.

S. A. MacKenzie, T. A. Kegelman and M. P. DeLorme. *Inhalation Toxicology, DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE.*

Acetylated lecithin (AcL) is a commonly used emulsifying agent; however, no safety assessment information exists for this test material by the inhalation route. The purpose of this study was to determine the acute inhalation effects of AcL in rats. Due to the viscous nature of AcL a respirable atmosphere of appreciable concentration could not be generated. To facilitate atmospheric generation, AcL was diluted 50:50 (v:v) with corn oil. Therefore, this study also assessed the acute inhalation safety of corn oil. Two groups of 5 male and 5 female rats each, were exposed nose-only for a single 4-h period to either corn oil or a mixture of AcL+corn oil. The atmospheres were generated by aerosolizing the test substances with nebulizers and airborn aerosol concentrations determined by gravimetric analysis. Filters collected from the AcL+corn oil atmosphere were also analyzed by HPLC to quantitate AcL concentration. Following exposure, animals were weighed and observed for clinical signs of toxicity during a 14-d recovery period. Rats were exposed to 2.5 ± 1.2 mg/L corn oil (mean ± SD) which was characterized by a MMAD of 1.9 µm ± 2.1 (± GSD). All rats exposed to the corn oil survived the exposure, demonstrated no loss in body weight, no clinical signs of toxicity and gross pathological examination revealed no evidence of organ-specific toxicity. A second group of rats was exposed

to 2.9 ± 0.62 mg/L AcL+corn oil, the maximum practically attainable atmospheric aerosol concentration, which was characterized by a MMAD of 3.3 μm ± 2.5. HPLC analysis of samples collected from the 2.9 mg/L atmosphere indicated that animals were exposed to 0.89 ± 0.036 mg/L AcL and 1.8 ± 0.63 mg/L corn oil. All rats exposed to 2.9 mg/L AcL+corn oil survived the exposure, demonstrated no loss in body weight, no clinical signs of toxicity and gross pathological examination revealed no evidence of organ-specific toxicity. Therefore, under the conditions of this study, the 4-h LC50 for AcL was greater than 0.89 mg/L, the maximum practically attainable atmospheric concentration.

PS 586 6:2 FLUOROTELOMER ALCOHOL: INHALATION EXPOSURE ATMOSPHERE CHARACTERIZATION AND ACUTE INHALATION TOXICITY.

D. P. Kelly, T. L. Serex, R. C. Buck, S. E. Loveless and M. P. DeLorme. *Inhalation Toxicology, DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE.*

6:2 Fluorotelomer Alcohol (CAS# 647-42-7, 1-Octanol-3,3,4,4,5,5,6,6,7,7,8,8,8 tridecafluoro-,6:2 FTOH) is a raw material used in the manufacturing of surfactant and polymeric surface protection products. This study was undertaken to develop 6:2 FTOH analytical and aerosol generation methods for determination of acute inhalation toxicity in rats. The vapor pressure and boiling point (0.33 mmHg and 172° C) of 6:2 FTOH resulted in vapor and aerosol phases at STP with higher atmospheric concentrations. The maximal vapor exposure concentration that could be readily achieved under conditions suitable for animal exposures before the aerosol phase of 6:2 FTOH began to form in the exposure atmosphere was 1.7 mg/L (110 ppm). In the toxicity determination, groups of 5 male and 5 female rats each were exposed nose-only for a single 4-hr period to 6:2 FTOH concentrations of 0.65, 1.7, 5.2 or 9.9 mg/L generated by either flash evaporation (0.65, 1.7, and 5.2 mg/L) or nebulization (9.9 mg/L). At the 0.65 and 1.7 mg/L exposures, all of the test substance was in the vapor phase. The 5.2 and 9.9 mg/L atmosphere contained 3.6 and 2.9 mg/L (240 and 190 ppm v/v) test substance in the vapor phase, respectively. Exposure concentrations were determined by gas chromatography and gravimetric analysis for the vapor and aerosol phases, respectively. The mean mass median aerodynamic diameters (MMAD) determined for the aerosol component from 2 samples collected during each of the 5.2 and 9.9 mg/L exposures were 6.5 and 3.8 μm, respectively. All rats exposed to 9.9 mg/L demonstrated labored breathing, weight losses >10%, and died within 6-days following exposure. Discoloration of the lungs was present in most of the animals exposed to 9.9 mg/L. All animals survived following the 0.65, 1.7 and 5.2 mg/L 6:2 FTOH inhalation exposures. Therefore, under the conditions of this study, the 4-hour inhalation median lethal concentration (LC50) for 6:2 FTOH was > 5.2 mg/L but < 9.9 mg/L.

PS 587 6:2 FLUOROTELOMER ALCOHOL: ONE-WEEK INHALATION TOXICITY STUDY.

M. P. DeLorme, T. L. Serex, S. R. Frame, R. C. Buck and S. E. Loveless. *Inhalation Toxicology, DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE.*

6:2 Fluorotelomer Alcohol (CAS# 647-42-7, 1-Octanol-3,3,4,4,5,5,6,6,7,7,8,8,8 tridecafluoro-,6:2 FTOH) is a raw material used for manufacturing surfactant and polymeric surface protection products. Inhalation of the 6:2 FTOH vapor phase is a relevant route of exposure for toxicity determination. In this 1-wk study, 4 groups of 5 male and 4 groups of 5 female rats were exposed whole body for 6 hrs/d for 5 d to 0 ± 0, 1.4 ± 0.22, 10 ± 0.16 or 96 ± 1.4 ppm 6:2 FTOH (mean ± SEM). No clinical signs of toxicity were observed in this study at any concentration. Rats exposed to 96 + 1.4 ppm 6:2 FTOH demonstrated statistically significant reductions in body weight of 8% on day 5 for males and 6-8% for females on days 1, 3, and 5. The magnitude of these body weight reductions were less than 15% and therefore considered to be test substance-related but non-adverse. Test substance-related changes in organ weights in both male and females exposed to 96 + 1.4 ppm included decreased thymic weights and males demonstrated increased mean liver weights relative to both body and brain weights; however, they were not associated with correlative microscopic changes and were considered non-adverse. Microscopic findings were limited to the incisor teeth of male and female rats exposed to 96 + 1.4 ppm 6:2 FTOH observed as basophilic striations within the inner dentin. This change is a common finding in rats following exposure to fluorine-containing compounds, was not associated with changes in odontogenic epithelium and was considered non-adverse. No effects on body weights, organ weights or microscopic changes were observed at lower concentrations. Therefore, under the conditions of this study, the NOAEL for 6:2 FTOH is 96 + 1.4 ppm. These findings correlate with the target organ effects observed in oral studies with 6:2 FTOH and indicate a lack of a route of entry effect. Therefore route to route extrapolation for systemic effects is appropriate for risk assessment of this material.

PS 588 BEHAVIORAL EFFECT OF CHRONIC INHALATION EXPOSURE TO MOTORCYCLE EXHAUST IN RATS.

H. Yu, T. Li and T. Ueng. *National Taiwan University, Taipei, Taiwan.*

The exhaust from motorcycles (scooters) is a major source of air pollutants in urban areas where motorcycles are a popular means of transportation. Motorcycle exhaust (ME) from two-stroke engines contains higher levels of toxic agents and chemical carcinogens than the exhaust from diesel engines. ME produces oxidative stress, DNA damage, inflammation, and enzyme induction in liver and lung cell lines and induces pulmonary and testicular toxicities in rats. The purpose of the present study was to explore the ability of ME inhalation exposure to cause behavioral effects in rats. Female Wistar rats were exposed to 1:10 diluted ME by inhalation from 9 to 10 am and 4 to 5 pm daily, Monday through Friday, for 8 weeks. Control rats were exposed to clean air. ME exposure increased immobility behavior in the forced swim test for depressant-like effects. The exposure decreased transition number and reduced time spent in the light area in the light-dark transition assay for anxiety-like behaviors. ME resulted in an increase of latency time in the tail-flick test for nociceptive effects. Serum acetylcholinesterase activity and brain 5-hydroxytryptamine 2A receptor mRNA expression in ME-exposed rats were lower than the respective controls. These preliminary findings indicated that chronic ME inhalation exposure has the ability to cause depression-, anxiety-, and antinociception-like behavioral effects in female rats.

PS 589 STUDY OF GENERATING AND MONITORING PROTEIN AEROSOL FOR INHALATION STUDY.

J. Song, H. Yang, S. Kwon, K. Jung, Y. Yang, K. Lee, J. Heo, C. Kim and C. Song. *Koeran institute of Toxicology, Jeong-up, Republic of Korea.*

There are increasing trials to deliver protein therapeutics using inhalation route, because of direct administering to pulmonary disease lesion, rapid drug absorption due to large surface area and good vasculization, and non-invasive nature. For assessing the efficacy or toxicity of the protein drugs which will be administrated via inhalation, it is of great importance to set the conditions target concentration of protein aerosol is generated in. In order to establish generation and monitoring method of protein molecule, bovine serum albumin (BSA) solution was chosen and aerosol was generated using mist generator (syringe pump type). diverse syringe pump speed (50, 100, and 200 μl/min) and stock solution (0.2, 0.5, 1, 2.5, 5, and 20 mg/ml) were tried. To monitor the concentration of the protein aerosol, samplers with filter were placed at the animals' breathing zone in an inhalation chamber. Sampled protein was eluted in distilled water and the concentration of aerosol was measured with BCA assay. And the particle-size distribution was determined with Scan Mobility Particle Sizer at the same position. BSA concentrations in the atmosphere increased proportionally to stock concentration and syringe speed. The proportionality of BSA concentration was more direct in higher concentration of BSA stock than in lower stock. Aerosol diameter and aerosol number concentration also increased according to the concentration of stock solution and the syringe pump speed. Based on these results, the target concentration can be calculated considering stock concentration and syringe speed. For the further study, generation and measuring low concentration of protein aerosol in diverse protein with different size will be needed

PS 590 A MOUSE VIDEO INSTILLATOR (MOVI) FOR INTRATRACHEAL INSTILLATION IN THE MOUSE.

K. Lee[1], B. Lee[2], K. Cho[1], C. Kim[1] and C. Song[1]. *[1]Korea Institute of Toxicology, Jeongup, Republic of Korea and [2]Doobae System, Seoul, Republic of Korea.*

Intratracheal instillation (ITI) of a test compound to rodent is difficult and the variation of the results is very big and various with respect to the skill of experimenters. Auto video instillator for rat was developed and validated in our previous study. At this time we intended to develop a new instillator for mouse because of its wide applications in a various research area, such as alternative inhalation exposure, pulmonary disease model, etc. We redesigned and reconstructed a 'MOVI" (Mouse Video Instillator) for the accurate delivery of a dose of a test compound into the trachea of mouse. The device has a videocamera probe basically for image guidance,

therefore more accurate and reproducible instillation is possible. And this overcomes some problems occurred in the adopting process of previous model to mice. To validate this, instillation of Evance Blue dye and induction of lung fibrosis with Bleomycin have been performed. The instilled dye dispersed well on the whole lungs and the variation of fibrosis prognosis was low. Based on the results of these examinations, we concluded that the device could be used for rapid, reproducible and successful ITI of a test compound into the lungs of mice.

PS 591 *IN VITRO* AND *IN VIVO* ANALYSIS OF SIZE-FRACTIONATED PM FROM DIFFERENT U.S. CITIES.

C. A. Hickey, L. Horton, K. Galdanes, M. Lippmann and T. Gordon.
Environmental Medicine, New York University School of Medicine, Tuxedo, NY.

A strong association between ambient PM and adverse cardiopulmonary health effects has been consistently reported. As PM toxicity has shown differences depending on particle size, season, and location, it has become clear that mass concentration alone may not be the best indicator of PM-induced health effects. We hypothesized that the differences in the PM composition account for these varied effects. A high volume cascade impactor was used to collect over 350 size-fractioned PM samples from five U.S. cities in 2007-08. In vitro analysis was conducted using a human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) and a human bronchial epithelial cell line (BEAS-2B). Results show size and seasonal effects on reactive oxygen species (ROS) formation (as measured by oxidation of a fluorescent dye) when data is separated by city. For example, winter coarse samples from Anaheim, CA elicited a greater production of ROS than the corresponding summer samples, while the opposite was true for the fine and ultrafine samples. Interestingly, both cell types did not respond similarly in all instances suggesting they have different sensitivities and response pathways. Following 6 and 24 h treatments with PM, mRNA expression levels for markers of ROS and cellular inflammation were measured by real-time RT-PCR. Results show differences in expression depending on size, city and duration of exposure. The most notable expression changes were observed for HO-1 and IL-8. Additionally, a subset of samples was analyzed in a murine model using oropharyngeal aspiration of PM. PMN levels measured in BAL did not correlate with in vitro ROS production suggesting the ROS production may not be the best indicator of PM toxicity in vivo. Our results support the hypothesis that the elemental composition of PM drives PM-induced health effects as indicated by differences in response depending in size, city and location. Future studies aim to correlate these findings with individual elemental data and source apportionment.

PS 592 TOBACCO SMOKE MODULATES THE PULMONARY AND CNS EFFECTS OF OZONE INHALATION IN RATS.

V. Bhoopalan[1], M. M. Shah[1, 3], D. M. Thomas[1, 3], S. Han[2] and D. K. Bhalla[1].
[1]Pharmaceutical Sciences, Wayne State University, Detroit, MI, [2]Animal and Food Sciences, University of Kentucky, Lexington, KY and [3]R&D Service, John D. Dingell VAMC, Detroit, MI.

Ozone (O3), an oxidant air pollutant, and tobacco smoke (TS) are known risk factors in the development of lung injury and chronic disease. While the independent toxicity of these pollutants has been widely studied, their interactive effects are poorly defined. This study was performed to determine if sequential exposures of rats to O3 and TS will influence the toxic responses of O3 in the lungs and brain. Adult Sprague Dawley (SD) male rats were exposed for a single 3 hr period to O3, TS or O3 plus TS in sequence. In all, 3 exposure groups were evaluated: 1) Air (control), 2) O3, 3) O3 followed by TS (O3/TS). Bronchoalveolar lavage (BAL) was performed, and BAL cells and fluid were analyzed. Data revealed a significant increase in polymorphonuclear leukocytes (PMN) and total BAL protein in the O3 group compared to the control, reflecting the inflammatory and cytotoxic effects of O3. However, a subsequent exposure to TS attenuated PMN infiltration into the air spaces for recovery in the BAL of the O3/TS group. A similar reduction was also observed for BAL protein in the O3/TS group, but it was not significant. Since O3 also induces deleterious effects in the brain, including the nigrostriatal pathway and because the dopaminergic cells of this region are thought to be hypersensitive to oxidative stress, we measured striatal dopamine levels in the O3 and O3/TS groups by HPLC with electrochemical detection. Data revealed O3 reduced striatal dopamine content, while the subsequent TS exposure afforded a nonsignificant protection. Overall, the results show that the toxicity of O3 in the lung and brain are modulated by exposure to TS. The results are contrary to the synergistic toxicity predicted for TS and O3, and are comparable to the attenuated responses following sequential exposures to carbon nanotubes and O3 reported in our previous studies. Supported in part by funding from OVPR-WSU, NIDA and VA.

PS 593 CYTOPROTECTIVE GENE EXPRESSION BY ACTIVATION OF NRF2 TRANSCRIPTION FACTOR IN LUNG OF RATS EXPOSED TO FINE AND ULTRAFINE AMBIENT PARTICULATE MATTER IN MEXICO CITY.

A. Valdés-Arzate[1], M. Uribe-Ramirez[1], O. G. Aztatzi-Aguilar[1], M. Tinoco-Mendez[2], C. Gómez-Ruiz[2], I. Gracia-Mora[3], M. T. Kleinman[4] and A. De Vizcaya-Ruiz[1]. *[1]Department of Toxicology, Cinvestav, Mexico City, Mexico, [2]Unidad de Experimentación Animal, Facultad de Quimica, UNAM, Mexico City, Mexico, [3]Departamento de Quimica Inorganica y Nuclear, Facultad de Quimica, UNAM, Mexico City, Mexico and [4]Division of Occupational and Environmental Medicine, Department of Medicine, University of California Irvine, Irvine, CA.*

Exposure to particulate matter (PM) has been associated with adverse health effects possibly initiated by oxidative stress within affected cells. The Nrf2 transcription factor, a master regulator of basal and inducible expression of detoxification and antioxidant enzymes such as superoxide dismutase (SOD-2), glutathione-S-transferases (GST) and heme oxygenase-1 (HO-1), can be activated in response to this type of stimuli. The aim of this study was to evaluate the cytoprotective response induction of Nrf2 from the exposure to PM in Mexico City. Sprague-Dawley male rats were randomly assigned into 4 groups (n=6) and exposed to coarse (C), fine (F) ultrafine (UF) or filtered air (FA) using a particle concentrator for 3 days and 8 weeks. Activation of Nfr2 in the lung was assessed by electrophoresis mobility shift assay (EMSA) and immunohistochemistry localization, and mRNA contents of HO-1, SOD-2 and GST were determined using RT-PCR. Our results show that F and UF PM increases Nrf2 nuclear translocation and ARE-binding in comparison to FA and C exposure groups, in the lung, leading to increased expression of SOD-2, GST and HO-1 genes at 3 days and 8 weeks. Thus, the acute (3 day) and subchronic (8 week) exposure to F and UF particles induce a significant coordinated induction of cytoprotective genes (HO-1, SOD-2 and GST) through activation of Nrf2 confering an adaptive survival response against toxicity caused by ambient F and UF deposited in the lung suggesting that Nrf2-activation is a key regulator of antioxidant defense responses that protects against pulmonary oxidative damage caused by PM. (Project financed by: CONACyT #57752 and UCLA-Fogarty Program).

PS 594 DETERMINATION OF HAZARD ASSOCIATED WITH AMINE AND AMINE DEGRADATION PRODUCTS FROM CARBON SEQUESTRATION PROCESSES.

V. Naik[1], J. McDonald[1], M. Doyle-Eisele[1], E. Knipping[2] and A. Rohr[2].
[1]Lovelace, Albuqueruque, NM and [2]Electric Power Research Institute, Palo Alto, CA.

There is currently an unmet need in understanding potential unintended health effects of exposure to amines that are utilized for CO2 capture in industrial operations. Because the processes utilize large volumes of amines, there is a chance for "slip" of these compounds and emission to the atmosphere. Furthermore, because the amines are utilized under highly oxidative conditions, there is potential for emissions of their degradation products. An experimental system has been developed to study the inhalation hazard of these amines and their degradation products. Monoethanolamine (MEA), a representative amine that may be used in this process, was used in initial studies conducted to assess potential hazard. The gaseous head space was obtained from the canister and delivered to a whole-body inhalation system or a reaction canister. Initial inhalation exposures to MEA were conducted at 18 mg/m3. Exposures were conducted in C57bl/6 mice 6h/day for 7 days. Toxicity was assessed by lavage counts/differentials, cytokine upregulation, and oxidative injury. The response to MEA atmosphere is compared with MEA that is degraded under conditions that simulate carbon capture processes. This includes both the addition of heat to ~150°C and the addition of oxygen, carbon dioxide and NOx. The reactions are conducted in a slightly pressurized non-adsorbent stainless steel canister. Reaction products are analyzed by mass spectrometry. The mixture is subsequently diluted and used for inhalation toxicity studies that compare to the hazard of MEA. Initial hazard evaluation or MEA degradation products are benchmarked against MEA atmospheres, which showed s mild inflammation at MEA atmospheres ≥ the occupational exposure limit. Research supported by the Electric Power Research Institute.

PS 595 TRACHEOBRONCHIAL AIRWAY MORPHOMETRY FOR THE C57BL/6 MOUSE: IMPLICATIONS IN INHALED DOSIMETRY PREDICTIONS.

L. B. Mendez[1, 2], R. F. Phalen[2] and G. J. Ramirez[3]. *[1]Microbiology and Molecular Genetics, University of California, Irvine, Irvine, CA, [2]Medicine, University of California, Irvine, Irvine, CA and [3]East Los Angeles College, Monterey Park, CA.*

The availability of molecular and genetic tools has made the mouse the most common animal model for a variety of human diseases. Among the factors that will influence dose delivery into murine lungs during an inhalation toxicology study are

respiratory tract anatomy, lung physiology, and clearance mechanisms. Limited data have found significant differences in the airway morphometry of two mouse strains with different genetic backgrounds. Therefore, the objectives of this study are to: 1) generate tracheobronchial (TB) morphometry data for the C57Bl/6 mouse; and 2) compare obtained TB data with those reported for the Balb/c and B6C3F1 mouse strains. To accomplish this, silicone rubber replica lung casts were prepared in the intact thorax of both male and female C57Bl/6 mice. Each TB airway was assigned an unique binary number and the airway length, diameter and branching angle was measured. No differences were observed between male and female mice of similar body weight and age. However differences in airway length and diameter were observed among all three inbred mouse strains. These differences have implications to the health outcomes during an inhalation study and in the extrapolation from animal toxicological data to humans.

PS 596 DECREMENTS IN HEART RATE VARIABILITY IN A ATHEROSCLEROTIC MOUSE MODEL DUE TO EXPOSURE TO SEMI-VOLATILE COMPONENTS OF ULTRAFINE CONCENTRATED AMBIENT PARTICULATES.

A. J. Keebaugh[1], G. Gookin[1], M. MacKinnon[1], K. Salazar[1], L. Mendez[2], D. Meacher[1], C. Sioutas[3] and M. Kleinman[1]. *[1]Medicine, University of California, Irvine, Irvine, CA, [2]Microbiology and Molecular Genetics, University of California, Irvine, Irvine, CA and [3]Civil and Environmental Engineering, University of Southern California, Los Angeles, CA.*

Increased levels of concentrated ambient particulates (CAPs) are associated with adverse cardiovascular effects including morbidity and mortality. Ultrafine particles (diameter <0.18 micrometers) are especially dangerous because they contain high concentrations of semi-volatile organic compounds (SVOCs) and have been associated with blood vessel inflammation that can result in heart disease. The primary objective of this project is to determine the contribution of semi-volatile components of ultrafine CAPs to changes in heart rate variability, a measure of cardiovascular function. Genetically altered mice susceptible to atherosclerosis (ApoE -/-) were exposed to ultrafine concentrated ambient particulates and to non-volatile particles from which the semi-volatile constituents had been eliminated by a thermal desorption system in series with a particle concentrator. The mice were exposed to CAPs and non-volatile particles for 5 hours a day, 4 days a week over an 8 week period during which another group of control mice were exposed to purified, filtered air. A telemetry device was surgically implanted to transmit electrocardiograph (ECG) readings from the mice during exposure and non-exposure periods over the 8 weeks. Heart rate variability was extracted from the ECG readings for the exposures, the night subsequent to the exposure, and similar time periods on days with no exposures. Significant decreases in heart rate variability were seen in the mice exposed to CAPs when compared to the mice exposed to non-volatile particles or the mice exposed to clean air. Due to the decrease in heart rate variability, it was concluded that vapor phase components of ultrafine ambient particles contribute to the adverse effects on cardiovascular function.

PS 597 DERIVATION OF PHYSIOLOGICAL INHALATION RATES IN CHILDREN, ADULTS, AND ELDERLY BASED ON NIGHTTIME AND DAYTIME RESPIRATORY PARAMETERS.

P. Brochu, K. Krishnan and J. Brodeur. *Université de Montréal, Montreal, QC, Canada.*

The methodology developed in our previous studies (Brochu *et al.* 2006a, b, c) for the determination of physiological daily inhalation rates was improved by integrating into the calculation process, both nighttime and daytime respiratory parameters, namely oxygen uptake factors (H) and ventilatory equivalents (VQ). H values during fasting (0.2057 ± 0.0018 L of O_2/kcal; mean ± S.D.) and postprandial phases (0.2059 ± 0.0019 L of O_2/kcal) as well as VQ values for subjects at rest (27.4 ± 4.8 to 32.2 ± 3.1, unitless) and during the aggregate daytime activities (29.9 ± 4.2 to 33.7 ± 7.2) were determined and combined with published doubly labeled water measurements for the calculation of daily inhalation rates in normal-weight males and females aged 0.22 to 96 years (n=1235). Depending upon the unit value chosen, the highest 99th percentiles for inhalation data were found in males aged 35 to less than 45 years (35.40 m^3/day), 2.6 to less than 6 months (1.138 m^3/kg-day) and 10 to less than 16.5 years (22.29 m^3/m^2/-day). Means and percentiles expressed in m^3/kg-day as well as m^3/m^2/-day suggest generally higher intakes of air pollutants in children than in adults and in males than in females (in μg/kg-day or μg/m^2-day) for identical exposure concentrations and conditions. For instance, means in boys aged 2.6 to less than 6 months of 10.99± 3.50 m^3/m^2/-day and 0.572 ± 0.191 m^3/kg-day are 1.3 and 2.5 fold higher, respectively, than those in adult males 65 to 96 years old (8.42 ± 2.13 m^3/m^2/-day, 0.225 ± 0.059 m^3/kg-day).

PS 598 DERIVATION OF CARDIAC OUTPUT AND ALVEOLAR VENTILATION RATE BASED ON ENERGY EXPENDITURE MEASUREMENTS IN HEALTHY MALES AND FEMALES.

B. Pierre, K. Krishnan and J. Brodeur. *Université de Montréal, Montreal, QC, Canada.*

Published indirect calorimetry measurements (n=902) and the disappearance rates of oral doses of deuterium (^2H) and heavy-oxygen (^{18}O) in urine monitored by gas-isotope-ratio mass spectrometry for an aggregate period of over 14, 000 days in the same subjects were used to determine respiratory and cardiovascular key parameters in healthy normal-weight males and females aged 5 to 96 years during their aggregate daytime activities. Arterioveinous oxygen content differences (0.051 to 0.082 ml of O_2 consumed/ml of blood) and ratios of the physiological dead space to the tidal volume (0.232 to 0.419) were determined for oxygen consumption rates (0.157 to 0.806 L/min) required by minute energy expenditures ranging from 0.76 to 3.91 kcal/min. Generally higher values for the 2.5th up to 99th percentile minute ventilation rates (0.132 to 0.774 L/kg-min, 4.42 to 21.69 L/m^2-min), alveolar ventilation rates (0.093 to 0.553 L/kg-min, 3.09 to 15.53 L/m^2-min), cardiac outputs (0.065 to 0.330 L/kg-min, 2.17 to 9.46 L/m^2-min) and ventilation-perfusion ratios (1.12 to 2.16) were found in children and teenagers aged 5 to less than 16.5 years compared to those for older individuals (0.076 to 0.461 L/kg-min, 2.80 to 16.99 L/m^2-min; 0.047 to 0.312 L/kg-min, 1.73 to 11.63 L/m^2-min; 0.045 to 0.201 L/kg-min, 1.63 to 7.24 L/m^2-min and 0.78 to 2.40 respectively). Therefore, frequently higher uptakes of liposoluble air pollutants (in μg/L of blood) by the respiratory tract in the former compared to the latter age group are expected for chemicals that have identical absorption rates and behaviours in the body for all ages.

PS 599 EFFECT OF ETHANOL VAPORS, OZONE, AND NANOPARTICLES ON PULMONARY INFLAMMATION IN A RAT MODEL OF ALLERGIC ASTHMA.

A. Scarino[1], R. Tardif[2], P. Renzi[2] and M. Charbonneau[1]. *[1]INRS-Institut Armand-Frappier, Université du Québec, Laval, QC, Canada and [2]Université de Montréal, Montreal, QC, Canada.*

Epidemiological studies suggest that ozone, an important urban air contaminant, can increase asthmatic symptoms in exposed populations. Nanoparticles and ethanol vapors are air contaminants of recent concern. We have shown that ethanol vapors do not increase pulmonary inflammation in a rat model of allergic asthma, while effects of nanoparticles in this model are unknown. This study aimed to investigate pulmonary inflammation, at the cellular and cytokine level, of inhaled TiO2 nanoparticles, ozone, and mixtures with ethanol vapors in asthmatic rats. Brown Norway rats were sensitized (sc) and challenged (15 min inhalation, 14 d later) with 1.5% chicken egg ovalbumin (OVA); inflammation results from the OVA challenge. Leukocytes counts (mostly eosinophils) in bronchoalveolar lavages (BAL) were performed 48 h following the challenge. Full body exposure to ozone (0.4 or 1 ppm), TiO2 nanoparticles (9.35 mg/m3) and ethanol vapors (3000 ppm) were done in a 500 L inhalation chamber. Using the Rat Cytokine Array Panel A (R&D Systems), the level of 29 cytokines was measured in pooled BAL supernatant. In OVA challenged rats, exposure to ozone (4 h/d, 3 consecutive days starting on challenge day) showed a 3.3-fold decrease in eosinophils compared to controls. Similarly, levels of cytokines known to be involved in asthmatic response, such as IL-13 (6-fold) and MIP-1α (4-fold), were decreased. Co-exposure to ethanol vapors did not change the response caused by ozone alone. In OVA challenged rats, exposure to TiO2 nanoparticles (6 h, single exposure) also decreased the eosinophil count compared to controls. Both ozone and TiO2 nanoparticles reduced the increase of leukocytes and cytokines caused by the OVA challenge. Thus, data obtained using a rat model of allergic pulmonary inflammation suggests a protection when the contaminants are administered subsequently to the induction of the inflammation. Ethanol vapors did not modify the response. (supported by AFSEET)

PS 600 QUANTIFICATION OF THE PATHOLOGICAL RESPONSE AND FATE IN THE LUNG AND PLEURA OF CHRYSOTILE IN COMBINATION WITH FINE PARTICLES COMPARED TO AMOSITE-ASBESTOS FOLLOWING SHORT-TERM INHALATION EXPOSURE.

D. M. Bernstein[1], R. A. Rogers[2] and H. E. Stewart[3]. *[1]Consultant in Toxicology, Geneva, Switzerland, [2]Rogers Imaging Corporation, Needham, MA and [3]Georgia-Pacific, LLC, Atlanta, GA.*

The marked difference in biopersistence and pathological response between chrysotile and amphibole asbestos has been documented in studies on chrysotiles from a range of sources. This study, however, is unique in that it has examined a

commercial chrysotile product that was used as a joint compound and that it has quantified not only the pathological response and fiber distribution by compartment in the lung but also using non-evasive techniques the translocation of fibers to and pathological response in the pleural cavity. This presentation presents the final results from the study and in particular the details of the quantification of measurements of fibers and pathological response in the pleural cavity.
Rats were exposed by inhalation 6 h/d for 5 days to a well-defined fiber aerosol. Sub-groups were examined through 1-year post-exposure. The translocation to and pathological response in the pleura was examined by SEM and confocal microscopy (CM) using non-invasive methods. The number and size of fibers was quantified using TEM and CM. This is the first study to employ such techniques to characterize fiber translocation to and the response of the pleural cavity. Amosite fibers were found to remain partly or fully imbedded in the interstitial space through 1 year post exposure and quickly produced granulomas (0 days) and interstitial fibrosis (28 days). Amosite fibers were observed penetrating the visceral pleural wall and were found on the parietal pleural within 7 days post exposure with a concomitant inflammatory response seen by 14 days post exposure. Pleural fibrosis and adhesions were also observed in response to the amosite. No cellular or inflammatory response was observed in the lung or the pleural cavity in response to the chrysotile and sanded material exposure. These results provide confirmation of the important differences between chrysotile mixed with sanded joint compound and amphibole asbestos.

PS 601 TWO-WEEK INHALATION EXPOSURE OF RATS TO LIBBY AMPHIBOLE (LA) AND AMOSITE ASBESTOS.

S. H. Gavett[1], A. M. Jarabek[1], E. W. Tewksbury[2], E. Bermudez[2], V. A. Wong[2], E. A. Gross[2], G. A. Willson[3], H. G. Wall[3] and D. E. Dodd[2]. *[1]U.S. EPA, Research Triangle Park, NC, [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC and [3]Experimental Pathology Laboratories, Inc., Research Triangle Park, NC.*

The relative potency of LA compared to UICC amosite was assessed in a subacute inhalation study designed to set exposure levels for a future subchronic study. Male F344 rats (n=7/group) were exposed nose-only to air (control), 3 concentrations of LA, or 1 concentration of amosite 6 hr/d, 5 d/wk, for 2 wk. Target and actual mean mass (mg/m^3) concentrations were: air (0, 0.077); low (0.5, 0.56), medium (3.5, 3.52), and high (25, 28.23) LA; and amosite (3.5, 3.67). There were no overt signs of toxicity during the study. One day after exposure, mean % neutrophils in bronchoalveolar lavage (BAL) fluid correlated well with exposure levels: control 0.8%; low (2.1%), medium (10.5%), and high (47.2%) LA; amosite 7.4%. BAL fluid protein, lactate dehydrogenase (LDH), alkaline phosphatase, and N-acetyl-β-D-glucosaminidase were significantly increased in the high LA group compared to control or amosite. LDH and protein in the medium LA group were significantly higher than either air or amosite. Four days after exposure, separate groups of rats were evaluated for trachea and lung histopathology. The medium LA group had minimal to moderate alveolar inflammation which was slightly more severe than in the amosite group. Bronchiolar epithelial hyperplasia was observed in the high LA group. Minimal interstitial fibrosis was observed in the medium and high LA and amosite groups. Cell replication in terminal bronchioles assessed by BrdU was not consistently increased due to background focal inflammatory responses in 2 control and 2 low LA animals. Removal of these resulted in a LA concentration-related increase in cell replication. In contrast to prior intratracheal instillation studies (Padilla-Carlin et al., SOT 2009), inhalation exposure to the medium LA level caused equivalent or greater lung injury than a comparable concentration of amosite asbestos. (This abstract does not represent US EPA policy).

PS 602 LONG TERM RESPONSE OF RATS TO SINGLE INTRATRACHEAL EXPOSURE OF LIBBY AMPHIBOLE (LA) OR AMOSITE.

J. M. Cyphert[1], D. J. Padilla-Carlin[1], M. C. Schladweiler[2], A. Nyska[3], J. H. Shannahan[1], U. P. Kodavanti[2] and S. H. Gavett[2]. *[1]Curriculum in Toxicology, UNC School of Medicine, Chapel Hill, NC, [2]EPHD, NHEERL, U.S. EPA, Research Triangle Park, NC and [3]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.*

In former mine workers of Libby, Montana, exposure to amphibole-contaminated vermiculite has been associated with increased incidences of asbestosis and mesothelioma. In this study, we investigated long term effects of Libby amphibole (LA) exposure in a rat model. Rat respirable fractions of LA and amosite (aerodynamic diameter < 2.5 μm) were prepared by water elutriation. Male F344 rats were exposed to single doses of either saline, amosite (0.65 mg/rat), or LA (0.65 or 6.5 mg/rat) by intratracheal instillation. One year after exposure, blood and bronchoalveolar lavage fluid parameters were at control levels, consistent with inflammation in the lungs being specifically intra-alveolar and interstitial. Inflammation was characterized as predominantly macrophage accumulation near terminal and respiratory bronchioles. Two years post-exposure, no potential preneoplastic changes were seen pathologically or in lung biomarker evaluation of any groups. LA caused dose-related increases in multifocal intraalveolar and interstitial accumulation of macrophages and fibroblasts, but little collagen deposition was seen. Amosite caused interstitial nodules composed of fibroblasts and multinucleated giant cells, with significant increases in collagen deposition. Surprisingly, expression of fibrosis markers Col 1A1 and Col 3A1, while trending to increase in all asbestos-exposed groups, did not reach statistical significance in amosite exposed rats, suggesting that the fibrosis was no longer active. Conversely, both Col 1A1 and Col 3A1 were significantly elevated in LA-exposed rats indicating that, although no collagen deposition was present histologically at 0.65 mg/rat LA, fibrosis may be initiating at this later stage. These results show that there are significant differences in long term toxicity of LA and amosite administered at the same mass dose. (This abstract does not represent U.S. EPA policy).

PS 603 LIBBY AMPHIBOLE-INDUCED INFLAMMATION IS MODULATED BY IN IRON *IN VITRO* AND *IN VIVO*.

J. Shannahan[1], M. Schladweiler[2], J. McGee[2], J. Richards[2], S. Gavett[2], A. Ghio[2] and U. Kodavanti[2]. *[1]University of North Carolina-Chapel Hill, Chapel Hill, NC and [2]NHEERL, U.S. EPA, Research Triangle Park, NC.*

Complexation of host iron (Fe) to asbestos after exposure has been postulated as a mechanism of oxidative stress possibly contributing to *in vivo* pulmonary injury and variations in disease susceptibility. In a series of experiments, we examined the role of Fe in Libby amphibole (LA) induced toxicity *in vitro* and *in vivo*. In a cell free media, LA bound ≈ 16μg of Fe/mg of fiber and increased reactive oxygen species (ROS) generation ≈ 3 fold; which was reduced by deferoxamine (DEF) treatment. To determine the role of Fe in LA-induced ROS generation and inflammation, BEAS2B cells were exposed to LA (50μg), Fe-loaded LA, or LA with DEF. No conditions altered HO-1 or ferritin mRNA expression. LA markedly increased IL-8 expression, which was significantly reduced by Fe loading but increased by DEF. To determine the role of Fe in LA-induced lung injury *in vivo*, spontaneously hypertensive rats were exposed intratracheally to either saline (300μl), DEF (1mg), $FeCl_3$ (21μg), LA (0.5mg), Fe-loaded LA (0.5mg), and LA+DEF (0.5mg). Neither $FeCl_3$ nor DEF increased bronchoalveolar lavage fluid (BALF) neutrophils compared to saline at 24h. LA caused BALF neutrophils to increase dramatically. Loading of Fe on LA but not chelation slightly decreased inflammation (LA+DEF>LA> Fe-loaded LA). At 4h post-exposure LA-induced lung mRNA expression of MIP-2 was significantly reduced in rats exposed to Fe-loaded LA but increased by DEF (LA+DEF > LA > Fe-loaded LA). Ferritin mRNA expression was elevated in rats exposed to Fe-loaded LA compared to LA control. HO-1 expression was unchanged due to LA exposure. In conclusion, the acute inflammatory response to LA exposure might be modified by the fiber's ability to complex Fe rather than redox cycling of fiber associated Fe and thus, Fe overload conditions may influence susceptibility to LA-induced pulmonary disease (This abstract does not represent US EPA policy).

PS 604 INHALATION OF OZONE AND DIESEL EXHAUST PARTICLES (DEP) INDUCES ACUTE AND REVERSIBLE CARDIAC GENE EXPRESSION CHANGES.

U. P. Kodavanti[1], B. Vallanat[2], M. C. Schladweiler[1] and A. D. Ledbetter[1]. *[1]EPHD, NHEERL, U.S. EPA, Research Triangle Park, NC and [2]RCU, NHEERL, U.S. EPA, Research Triangle Park, NC.*

We have recently shown that episodic but not acute exposure to ozone or DEP induces vascular effects that are associated with the loss of cardiac mitochondrial phospholipid fatty acids (DEP 2.0 mg/m3 > ozone, 0.4 ppm). In this study we determined ozone and DEP-induced cardiac gene expression changes following acute and episodic exposures. Male WKY rats were exposed nose-only to air, ozone (0.4 ppm), resuspended DEP (2.0 mg/m3), or ozone+DEP, 5 h/d, 1 d/wk for 16 wk; or air, ozone (0.5 ppm), or DEP (2.0 mg/m3), 5h/d for 2 d. Left ventricular tissues were analyzed for gene expression using Affymetrix rat 2.0 arrays (n=4-6) 1 d after acute and 2 d after 16 wk exposure. Surprisingly, only minimal alterations were noted in rats exposed for 16 wk (ozone, 38; DEP, 165; ozone+DEP, 26 genes). Notable exposure-related effects were observed in rats in the acute exposure protocol (ozone, 1421 and DEP, 1517 genes; $p<0.05$), suggesting that cardiac gene expression changes were reversible and/or exhibited adaptation upon repeat exposures

as generally seen in functional analysis. Most gene changes in both exposures involved regulators of signaling, ion channels, oxidation/reduction reactions and immune function. More ion channel genes were affected by ozone than DEP suggesting greater physiological impact at concentrations used. Remarkable similarities existed in the direction of change (increase or decrease) between ozone and DEP. Few genes were differentially affected, however those genes are deemed critical in cardiac injury; e.g., sarcolipin induced by ozone but inhibited by DEP. Critical genes induced by ozone but not DEP included Apo E, PGD2, aldolase, epoxide hydrolase and adenosine monophosphate deaminase. More genes were inhibited by DEP when compared to ozone. These data provide mechanistic insights into the commonalities of air pollutant induced cardiovascular effects that may reflect acute and reversible physiological changes observed after exposure. (This abstract does not reflect USEPA policy).

PS 605 MODULATION BY NANO-SIZED CERIUM OXIDE OF THE TOXICITY OF INHALED DIESEL ENGINE EXHAUST IN ATHEROSCLEROSIS-PRONE APOLIPOPROTEIN E-DEFICIENT MICE.

F. R. Cassee[1], J. F. Boere[1], P. H. Fokkens[1], D. L. Leseman[1], P. Krystek[3], I. Gosens[1] and M. R. Miller[2]. *[1]Centre for Environmental Health, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom and [3]MiPlaza Material Analysis, Eindhoven, Netherlands.*

It is expected that there will be an increased exposure of a large population to cerium oxide (CeO2) nanoparticles used as fuel additive. Very little is known about the public health impacts of this new technology. ApoE mice were fed a Western diet for 8 weeks and during the last 4 weeks exposed to diluted diesel engine exhaust for 5 days/week. Exhaust was generated either from standard diesel (DE) or a diesel-spiked with 10 ppm cerium nanoparticles (DCeE), believed to improve the burning efficiency of the fuel and decrease particle number in the exhaust. Diluted diesel exhaust was given at 3 'doses' by having 3 different periods of exposure: 20 min (low), 1 h (medium) & 3 h (high) of 1.7 mg/m3 particle mass. Blood was taken to look at systemic inflammation and other markers and a range of organs were dissected to look for signs of gross pathology. In addition, the cross-sectional area of plaques throughout the brachiocephalic artery of each animal. Typical primary particle size of the nano-particles as seem with TEM-EDX was <5 nm. The addition of CeO2 resulted in a reduction of the number of particles (30%) as well as the surface area of the particles (10%), whereas the gaseous components slightly increased (6-8%) at the same particle mass concentration (1.7 mg/m3) and size distribution (80 nm). Despite these differences no clear signs of modulation of toxic effect due to DE were noted in lung, liver, heart, kidney or spleen. There was a trend towards increased severity of the atherosclerotic plagues due to DE whereas teh opposite was observed for DCeE. We conclude that from this study that, although cerium oxide may result in a change in the physicochemical composition of diesel engine exhaust, there is no need for concern towards increased toxicity of the mixture due to the use of cerium oxide.

PS 606 COMPARISON OF TOXICITY OF BIODIESEL *VERSUS* PETROLEUM-DIESEL-FUELED ENGINE EXHAUST IN AN ASTHMA MOUSE MODEL.

G. R. Gookin[1], A. Keebaugh[1], L. M. Mendez[2, 1], B. Haghverdian[1], D. Meacher[1], S. Nakao[3], D. R. Cocker[3] and M. T. Kleinman[1]. *[1]Medicine, University of California Irvine, Irvine, CA, [2]Microbiology and Molecular Genetics, University of California Irvine, Irvine, CA and [3]Chemical and Environmental Engineering, University of California Riverside, Riverside, CA.*

The use of alternative fuels, such as biodiesel, has resulted in emission of gases and particulate matter (PM) of different composition than standard diesel fuel. It is established that diesel exhaust particles are associated with the exacerbation of asthma and other respiratory diseases. Reduction in PM emissions from biodiesel combustion has led to the assumption of a reduction in pulmonary toxicity; however adequate toxicity testing has not been conducted to test this assumption. The primary objective of this project is to provide in vivo health effects data in an animal model of allergic asthma. To accomplish this objective, two aims were investigated: 1) the changes in pulmonary function of allergen sensitized mice exposed to 100% biodiesel exhaust (BDE) vs. clean air, 2) whether BDE exposure elicits significant differences in the aforementioned endpoints compared to petroleum based diesel exhaust (DE) exposure. To accomplish these aims, emissions from a diesel engine using 100% biodiesel or standard petroleum diesel at relevant ambient levels of NO, CO, O3, and PM were collected for toxicity testing in the ovalbumin senstitized Balb/c mouse, a commonly used animal model of allergic asthma. Emission data of BDE versus DE reflects a 3-fold increase in NO and a depression in PM, while CO and O3 levels remained equivalent. Mice instilled with PM from BDE and DE showed a depression in pulmonary function compared to saline controls. Airway hyperresponsiveness in allergic mice showed a significant increase evidenced by an increase in pulmonary resistance when exposed to either BDE or DE compared to controls. Lung compliance in both BDE and DE exposed mice decreased but not significantly. Initial results suggest that BDE exposure exacerbates asthmatic conditions at equal or greater levels than DE in an experimental asthma model.

PS 607 HYPOTENSION AND AV BLOCK AFTER DIESEL EXHAUST EXPOSURE IN HEART FAILURE-PRONE RATS: ROLE OF GASEOUS AND PARTICULATE COMPONENTS.

A. P. Carll[1], C. Lamb[2], M. S. Hazari[3], N. Haykal-Coates[3], D. W. Winsett[3], Q. T. Krantz[3], D. L. Costa[4] and A. K. Farraj[3]. *[1]Environmental Science & Engineering, University of North Carolina Chapel Hill, Chapel Hill, NC, [2]CURTOX, University of North Carolina Chapel Hill, Chapel Hill, NC, [3]Environmental Public Health Division, U.S. EPA, Research Triangle Park, NC and [4]ORD, U.S. EPA, Research Triangle Park, NC.*

Acute inhalations of diesel engine exhaust (DE) and fine particulate matter ($PM_{2.5}$) have been demonstrated to provoke adverse cardiac events in humans with preexisting heart disease. Electrophysiologic dysfunction and autonomic imbalance are among the mechanisms widely held to underlie the cardiac effects of DE and $PM_{2.5}$. We previously demonstrated that acute $PM_{2.5}$ inhalation (580 $\mu g/m^3$ metal sulfate, 4 hours) provokes cardiac arrhythmias, vagal dominance, delayed atrioventricular (AV) conduction, and systolic hypotension in young heart failure-prone hypertensive rats (SHHF strain) with cardiomyopathy. We hypothesized that inhalation of either whole (WDE: gases + PM) or filtered DE (FDE: gases alone), would similarly elicit cardiac dysfunction in naïve SHHFs. Unrestrained 10-week-old SHHFs were exposed once for 4 hours by inhalation to WDE (500 $\mu g/m^3$ $PM_{2.5}$), FDE, or filtered air (n=6-7/group). Both WDE and FDE decreased mean arterial pressure during exposure. Over the 8 hours following inhalation exposure, WDE- and FDE-exposed rats remained hypotensive, with effects more pronounced in the WDE group. WDE and FDE had minimal effects on heart rate variability, and pulmonary, cardiovascular, and systemic markers of inflammation, injury, and oxidative stress. FDE, but not WDE, significantly increased AV block Mobitz II arrhythmias after exposure relative to pre-exposure. Similar exposures in normal rats (Wistar Kyoto) failed to elicit bradyarrhythmias. Thus, predisposition to heart failure may confer susceptibility to the adverse effects of DE inhalation on cardiac function. This study demonstrates that gaseous and particulate components of air pollution may have divergent effects that are triggered by disparate mechanisms. *(Abstract does not reflect EPA policy; Supported by UNC/EPA CR83323601.)*

PS 608 A SINGLE EXPOSURE TO DIESEL EXHAUST INCREASES THE RISK OF TRIGGERED CARDIAC ARRHYTHMIAS IN CONSCIOUS RATS DURING DOBUTAMINE CARDIAC "STRESS" TEST.

M. S. Hazari, D. W. Winsett, N. Haykal-Coates, D. L. Costa and A. K. Farraj. *Environmental Public Health Division, U.S. EPA, Research Triangle Park, NC.*

Mild-to-moderate exercise is often used to stress the cardiovascular (CV) system of patients while monitoring them for electrocardiogram (ECG) abnormalities that may indicate underlying CV disease. We previously used dobutamine, which increases heart rate (HR) and contractility, to reveal exercise (stress)-induced cardiac electrical dysfunction in rats exposed to acrolein. In this study, we hypothesized that a single inhalation exposure to diesel exhaust (DE) would increase the risk of arrhythmia during the dobutamine "stress" test. Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats were surgically implanted with radiotelemeters and a chronic intravenous (iv) catheter in the left jugular vein. After recovery, rats were exposed whole-body to either air or 150 ug/m3 whole DE for 4hr. One day later, intravenous dobutamine (20, 40, 80, 160 and 320ug/kg) was administered to conscious rats while ventilatory parameters and ECG were monitored continuously. Dobutamine caused a dose-dependent increase in HR in air-exposed rats, this response was potentiated in WKY and SH rats exposed to DE. DE caused an increase in baseline HR and during challenge prevented recovery of resting HR. Exposure to DE significantly increased the number of atrial premature beats, ventricular premature beats, and non-conducted P-waves triggered by the challenge in both strains

relative to their controls. ST-depression was observed in all DE-exposed SH rats, but not WKY rats. In conclusion, the dobutamine stress test shows that rat strains with compromised cardiovascular systems are prone to elevated HR and arrhythmic events when compared to the healthy strain. A single exposure to a toxic air pollutant may increase the risk of triggering cardiac arrhythmias during exercise-like stress conditions. (This abstract does not reflect EPA policy)

PS 609 COMBINED EFFECTS OF DIESEL EXHAUST PARTICLES AND HIGH GLUCOSE ON MMP-2 EXPRESSION IN HUMAN MONOCYTES.

R. Wan[1], Y. Mo[1], S. Chien[2], D. J. Tollerud[1] and Q. Zhang[1]. [1]*Environmental and Occupational Health Sciences, University of Louisville, Louisville, KY and* [2]*Surgery, University of Louisville, Louisville, KY.*

A number of epidemiological studies and animal experiments have shown that individuals with preexisting diseases, such as asthma, chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), and cardiovascular diseases are more susceptible to particulate matter (PM)-related health problems. Macrophages are the first line of defense against inhaled particles and play an important role in PM-induced pulmonary and systemic inflammation and cardiac impairment. Hyperglycemia has been shown to activate macrophages/monocytes to secrete metalloproteinases which are involved in various hyperglycemia-induced cardiovascular diseases. However, few studies have focused on the activation of MMPs and the tissue inhibitors of MMPs (TIMPs) in macrophages/monocytes from DM subjects with exposure to diesel exhaust particles (DEP), one of the important components of PM, though several epidemiological studies have shown that people with DM were especially sensitive to effects of PM on daily mortality and heart disease hospitalization. In this study, we determined the combined effects of DEP with or without high glucose on human monocytes U937. Our results showed that exposure of U937 cells to DEP, high glucose or DEP with high glucose, at a non-toxic dose, induced reactive oxygen species (ROS) generation, increased phosphorylation of p38 and Erk1/2, and MMP-2 expression. Further, exposure to DEPs with high glucose caused significantly more ROS generation in U937 cells than those exposed to DEP or high glucose alone. Our results also showed that there were combined effects, including increased phosphorylation of p38 and Erk1/2 and upregulation of MMP-2 expression when U937 cells exposed to DEP with high glucose. Our results suggest that co-exposure to DEP and high glocuse causes synergistic injury to monocytes.
This work was partly supported by ALA(RG-872-N), AHA(086576D), KSEF-1686-RED-11, Health Effects Institute (4751-RFA-05-2/06-12), T32-ES011564.

PS 610 DISORDERED PORPHYRIN METABOLISM AMONG AUTISTIC CHILDREN IS NOT ASSOCIATED WITH MERCURY EXPOSURE.

J. S. Woods[1], S. E. Armel[1, 2], D. Echeverria[1, 3] and N. J. Heyer[3]. [1]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA,* [2]*Veritox, Inc., Redmond, WA and* [3]*Battelle Centers for Public Health Research and Evaluation, Seattle, WA.*

Increased urinary concentrations of pentacarboxyl-, precopro- and copro- porphyrins are associated with prolonged mercury (Hg) exposure in adults, and comparable increases have been attributed to Hg exposure in children with autism (AU). We compared urinary porphyrin concentrations in 2-12 year old neurotypical (NT) children (n = 59) and same age children with AU (n= 57) and examined the association between porphyrin levels and past or current Hg exposure. Caregivers provided information at enrollment regarding medical, dental and dietary Hg exposures. Urine samples from all children were acquired for porphyrin, creatinine and mercury analyses. We conducted logistic regression analysis to determine if porphyrin levels were associated with increased risk of AU. Mean urinary porphyrin concentrations are naturally high in young children and decline by as much as 2.5-fold between ages 2 and 12 years. Elevated copro- ($p<0.009$), hexacarboxyl- ($p<0.01$) and pentacarboxyl- ($p<0.001$) porphyrin concentrations were significantly associated with AU. No differences were found between NT and AU in mean[SD] urinary Hg levels (ug/g creatinine) (NT = 0.28[0.43]; AU =0.40[0.66]) or in past Hg exposures as determined by fish consumption, number of dental amalgam fillings, or vaccines received in total or prior to 2001. Logistic regression models of age-adjusted associations between porphyrin levels and AU vs NT indicated significant associations of hexacarboxyl-, pentacarboxyl- and copro- porphyrins with AU, with odds ratios[CI] = 1.65[1.07-2.55], 2.36[1.37-4.07] and 2.03[1.15-3.57], respectively. These findings define disordered porphyrin metabolism as a salient characteristic of autism. However, no differences in Hg exposures were found, and a porphyrin profile consistent with that seen in Hg-exposed adults was not apparent. Supported by the NIEHS (P30ES07033), the Autism Research Institute and the Wallace Research Foundation.

PS 611 PRENATAL EXPOSURE TO DIOXINS AND PCBS IS ASSOCIATED WITH INCREASED RISK OF WHEEZING AND INCREASED NUMBER OF UPPER AIRWAY INFECTIONS IN ONE YEAR OLD CHILDREN.

S. B. Stølevik[1], B. Granum[1], U. C. Nygaard[1], E. Namork[1], M. Haugen[1], H. E. Kvalem[1], H. M. Meltzer[1], J. Alexander[1], H. van Loveren[2, 3], J. H. van Delft[3] and M. Løvik[1, 4]. [1]*Norwegian Institute of Public Health, Oslo, Norway,* [2]*National Institute of Public Health and the Environment, Bilthoven, Netherlands,* [3]*Maastricht University, Maastricht, Netherlands and* [4]*Norwegian University of Science and Technology, Trondheim, Norway.*

During pregnancy, the foetus is exposed to dietary toxicants from the food consumed by the mother. We investigated if prenatal exposure to dioxins and polychlorinated biphenyls (PCBs) is associated with the occurrence of wheezing and upper respiratory tract infections in the first year of life.
Pregnant women were recruited to the birth cohort BraMat (n=205) under the EU-funded project NewGeneris. The occurrence of wheezing and upper respiratory tract infections in the children was assessed by questionnaire data at the age of one year (n=195). Prenatal exposure to the dietary toxicants was estimated from a validated food frequency questionnaire filled in by the mothers at midterm. The sum of six non-dioxin-like PCBs (PCB 28, 52, 101, 138, 153 and 180 (PCB6)) and the levels of dioxin and dioxin-like PCB toxic equivalents (total TEQ) were used. The intakes of PCB6 and total TEQ are expressed as ng/bw/day and pg/bw/week, respectively (adjusted for bodyweight before pregnancy). Logistic multivariate regression analyses were performed. The confounding variables included were gender, maternal history of atopy, maternal age, maternal BMI, maternal education, maternal smoking, parity, season of birth, breast-feeding of the child, mode of delivery and Apgar score.
Increased levels of PCB6 and total TEQ were both associated with a higher occurrence of wheezing (OR (95% CI); 2.79 (1.20, 6.49), p=0.018 and 3.35 (1.45, 7.74), p=0.005, respectively) and ≥7 episodes of upper respiratory tract infections (3.20 (1.20, 8.54), p=0.021 and 2.39 (0.88, 6.50), p=0.089, respectively). These results suggest that prenatal exposure to PCBs and dioxins from the mother's food intake affects the health status of one year old children.

PS 612 EVOLUTION OF MITOCHONDRIAL FUNCTION IN HEALTHY INFANTS *IN UTERO* EXPOSED TO HIV AND ANTIRETROVIRALS.

C. Morén[1], G. Garrabou[1], A. Noguera-Julian[2], N. Rovira[2], M. Nicolàs[1], E. Corrales[2], F. Cardellach[1], C. Fortuny[2] and <\s>Miró[1]. [1]*Mitochondrial Research Laboratory, Muscle Research Unit, Internal Medicine Department, IDIBAPS-University of Barcelona (Barcelona, Spain) and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, Valencia, Spain), Barcelona, Spain and* [2]*Infectious Diseases, Pediatrics Department, Hospital Sant Joan de Déu, University of Barcelona, Barcelona, Spain.* Sponsor: J. Domingo.

Background:Mitochondrial dysfunction of HIV-infection and antiretrovirals, especially nucleoside-reverse-transcriptse-inhibitors (NRTIs), has been widely associated to secondary effects in adults. Antiretrovirals are administered to HIV-infected pregnants to prevent vertical transmission. Little is known about the safety in their perinatally-exposed newborn. Objectives: To characterize mitochondrial function in peripheral-blood-mononuclear cells (PBMC) from healthy infants born to HIV-infected mothers and who were perinatally exposed to NRTIs. Methods: Cross-sectional, longitudinal case-control study in 2groups of healthy children: those perinatally exposed to HIV and NRTIs during pregnancy and the neonatal period (Cases,n=132) and those born to HCV-infected mothers (Controls,n=34). PBMC were obtained at 6weeks,3,6 and 12 months of life and prospectively cryopreserved. Mitochondrial content (citrate synthase activity) and CIV mitochondrial respiratory chain enzymatic activity were assessed spectrophotometrically. Results: Mitochondrial content remained similar in both groups along time. Compared to the control group, a decrease in CIV function was found in NRTIs and HIV-perinatally exposed infants at 6 weeks and 3 months of life (23.03±4.28 vs. 9.12±0.83 and 25.88±3.59 vs.9.62; $p<0.005$ and $p<0.001$, respectively). An increase in CIV activity was found in cases from 6weeks to 12months of life (9.12±0.83,vs.15.40±2.47;p=0.022). Conclusions: CIV activity was reduced in

NRTIs and HIV-perinatally exposed children compared to controls at 6 weeks and 3 months. Accordingly, a recovery in CIV function was found at 12months compared to 6 weeks in this group, suggesting reversibility in mitochondrial toxicity.

PS 613 ADVERSE PERINATAL OUTCOME AND MITOCHONDRIAL TOXICITY IN HIV-INFECTED PREGNANT WOMEN AND THEIR NEWBORN.

G. Garrabou[1], A. S. Hernandez[2], C. Moren[1], M. Lopez[2], M. Nicolas[1], O. Coll[2], F. Cardellach[1], E. Gratacos[2] and O. Miro[1]. [1]*Mitochondrial Research Laboratory, Internal Medicine Department, IDIBAPS-University of Barcelona, Hospital Clinic Barcelona (HCB, Barcelona, Spain) and Centro de Invest.Biomédica en Red de Enfermedades Raras (CIBERER, Valencia, Spain), Barcelona, Spain and* [2]*Materno-Fetal Medical Service, HCB, Barcelona, Spain., Barcelona, Spain.* Sponsor: J. Domingo.

Background: HIV-pregnant women show higher risk of adverse perinatal outcome. In non-pregnant adults, HIV and antiretrovirals cause mitochondrial toxicity and apoptosis responsible of secondary effects. We wonder if mitochondrial toxicity and apoptosis are present in HIV-pregnant women and their newborn and they could be the base of adverse perinatal events. Methods: We studied blood mononuclear cells (ficoll) of 27 HIV-infected and 31 uninfected pregnant women and their newborn at delivery. We measured apoptotic rate (active caspase-3/β-actin) and mitochondrial (mt) DNA content, mt protein synthesis (COX-II/V-DAC), mt function (enzymatic activities such as G3P+CIII) and mt number (citrate synthase) by western blot, rtPCR and spectrophotometry. Results: Adverse perinatal outcome was significantly increased in HIV-pregnancies (OR 7.33, 1.8-30.1, $p<0.005$). Mitochondrial amount was unaltered. However, the rest of mitochondrial parameters were reduced in HIV-mothers and their newborn, reaching significant differences: newborn mtDNA content, maternal and fetal mitochondrial protein synthesis and maternal G3P+CIII function (38.6%, 25.9%, 14.3% and 31.2% decreased, $p<0.05$). Materno-fetal mitochondrial parameters significantly correlated. Apoptosis was exclusively increased in HIV-women (p=NS). No correlation was found between adverse perinatal outcome and mitochondrial or apoptotic events. Conclusions: Transplacental ARV toxicity could cause the mitochondrial lesion of HIV-pregnant women and infants. Maternal apoptosis may be due to maternal-restricted HIV-infection. However, their implication in adverse perinatal outcome has been difficult to be established, probably due to the small size of our sample.
Financial support: FIPSE 360745/09.

PS 614 TOBACCO CAUSES MITOCHONDRIAL TOXICITY AND REDUCED BIRTH WEIGHT IN NEWBORN OF SMOKING PREGNANT MOTHERS.

F. Cardellach[1], G. Garrabou[1], A. S. Hernandez[2], C. Moren[1], M. Nicolas[1], O. Coll[2] and O. Miro[1]. [1]*Mitochondrial Research Laboratory, Internal Medicine Department, IDIBAPS-University of Barcelona, Hospital Clinic of Barcelona (HCB) and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, Valencia, Spain), Barcelona, Spain and* [2]*Materno-Fetal Medical Service, HCB, Barcelona, Spain, Barcelona, Spain.* Sponsor: J. Domingo.

Background: Fetuses exposed to tobacco show reduced intrauterine growth and weight at delivery of unknown etiology. It can be due to hypoxia induced by carbon monoxide (CO) binding to hemoglobin or to the mitochondrial toxicity of CO that binds mitochondrial complex IV (mCIV). Methods: We studied peripheral blood mononuclear cells (ficoll) of 30 smoking and 36 non-smoking pregnant women and their newborns at delivery. Mitochondrial CIV activity, mitochondrial amount, oxidative stress and tobacco consumption (cotinine) were measured by spectrophotometry and mitochondrial DNA (mtDNA) levels by quantitative rtPCR. Results: mCIV activity, absolute or relative to mitochondrial amount, and mtDNA were decreased in smoking pregnant mothers with respect controls (29.58±1.64 vs. 44.50±3.75, 0.26±0.01 vs. 0.35±0.02 and 2.82±0.29 vs. 3.54±0.41, $p<0.05$). Absolute mCIV and mtDNA content were significantly inhibited in their newborn (26.24±2.85 vs. 46.15±5.82 and 2.28±0.32 vs. 2.62±0.25, $p<0.01$ and $p<0.05$). Contrarily, oxidative stress was increased (p=NS) in those infants (0.65±0.08 vs. 0.60±0.04). Materno-fetal mCIV and oxidative stress were significantly correlated ($p<0.001$ and $p<0.05$) as well as their oxidative stress and newborn weight ($p<0.05$). Moreover, the tobacco consumed (cotinine) correlated with newborn weight at delivery, materno-fetal mCIV and maternal oxidative stress ($p<0.05$, $p<0.05$ and $p<0.01$). Conclusions: Mitochondrial dysfunction in both, smoking pregnant women and their newborns, indicates a common toxic etiology related to tobacco smoking. Additionally, the relationship among mitochondrial lesion, the amount of tobacco consumed and infant weight at birth suggests that CO or other toxic components of tobacco smoke could be the basis of reduced intrauterine growth.
Financial support: FIS 0229/08.

PS 615 MOLECULAR MECHANISMS REGULATING DRUG METABOLIZING ENZYME ONTOGENY.

R. N. Hines[1, 2], M. Le[1, 2], J. D. Shadley[1, 2], N. Giebel[1, 2], K. Dorko[3] and S. C. Strom[3]. [1]*Pediatrics & Pharmacology/Toxicology, Medical College of Wisconsin, Milwaukee, WI,* [2]*Children's Research Institute, Children's Hospital and Health Systems, Milwaukee, WI and* [3]*Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Differences in drug-metabolizing enzyme expression as a function of age have been well documented. Such differences clearly contribute to the differential susceptibility of children to chemical toxicity and adverse drug reactions. The molecular mechanisms controlling enzyme ontogeny and the high degree of interindividual variability are poorly understood. To address this problem, studies are being conducted to evaluate histone marks associated with chromatin structure within regulatory domains controlling *CYP3A4*, *3A7*, *FMO1* and *FMO3* expression. The relative enrichment of histone marks are determined using immunoprecipitation with pooled chromatin isolated from first trimester primary human fetal or adult hepatocytes and quantitation by RT-PCR. *CYP3A4* expression increases 20- to 100-fold between the third trimester and the adult. Evaluating the *CYP3A4* CLEM4 domain located from position -11,420 to -10,883 and containing USF1, HNF4α, HNF1α, and AP1 elements revealed a 6- to 14-fold enrichment of the H3K27me3 histone mark in fetal versus adult hepatocytes. In contrast, a 1.5- to 3-fold enrichment of the H3K4me1 histone mark was observed in adult versus fetal hepatocytes. A 100-fold difference in enrichment was observed in adult versus fetal hepatocytes for USF1. Similar results were observed for the presence of these same histone marks and the binding of C/EBPβ in the regulatory domain located between *CYP3A4* position -5900 and -5700. H3K27me3 enrichment in *CYP3A4* enhancer domains in the fetus is consistent with a role for condensed chromatin structure silencing expression while the H3K4me1 enrichment in these enhancer domains in the adult is consistent with a role for open chromatin structure promoting *CYP3A4* expression. These data are consistent with changes in chromatin structure having a dominant role in regulating *CYP3A4* ontogeny. Supported in part by PHS grant GM081344.

PS 616 PRENATAL EXPOSURE TO FLAVONOIDS: IMPLICATION FOR CANCER RISK.

K. Vanhees, L. de Bock, R. W. Godschalk, F. J. van Schooten and S. Barjesteh van Waalwijk van Doorn-Khosrovani. *Health Risk Analysis and Toxicology, Maastricht University, Maastricht, Netherlands.* Sponsor: H. van Loveren.

Flavonoids are potent antioxidants, freely available as high-dose dietary supplements. However, they can also induce DNA double-strand breaks (DSB) and rearrangements in the mixed-lineage leukemia (MLL) gene, which is frequently rearranged in childhood leukemia. We hypothesize that a deficient DSB-repair, as a result of an ATM-mutation, may reinforce the clastogenic effect of dietary flavonoids, increasing the frequency of MLL rearrangements. Therefore we examined the effects of in vitro and transplacental exposure to high, but biologically relevant amounts of the flavonoids genistein and quercetin in mice with different genetic capacity for DSB-repair (homozygous/ heterozygous knock-in for human Atm-mutation versus wild-type). In vitro exposure of bone marrow cells to genistein and quercetin induced higher numbers of Mll rearrangements in cells obtained from Atm-ΔSRI mutant mice compared to wild-type mice. Subsequently, female heterozygous Atm-ΔSRI mice were placed on a flavonoid-poor, genistein-enriched (270mg/kg feed) or quercetin-enriched (302mg/kg feed) diet throughout pregnancy. Offspring mice prenatally exposed to flavonoids had increased levels of Mll rearrangements, particularly those deficient in DSB-repair. Atm-ΔSRI mutant mice prenatally exposed to flavonoids also had an increased number of malignancies of different types already at 12-weeks of age (23% and 25 %, respectively for genistein and quercetin versus 2% in control mice). These data suggest that prenatal exposure to both genistein and quercetin supplements can increase the risk of malignancies, such as leukemia, especially in the presence of compromised DNA-repair.

PS 617 EFFECTS OF INTRAVENOUS AND ORAL ADMINISTRATION OF DEHP IN THE LUNGS OF NEONATAL MALE RATS.

J. R. Latendresse[2], L. Camacho[1], L. Muskhelishvili[2], S. J. Moon[1] and K. Delclos[1]. [1]*Division Biochemical Toxicology, National Center for Toxicological Research. Food and Drug Administration, Jefferson, AR and* [2]*Toxicologic Pathology Associates, Jefferson, AR.*

DEHP [di(2-ethylhexyl)phthalate] is a plasticizer commonly used in medical devices. While DEHP is best known as a rodent testicular toxicant, there are reports in the literature that indicate that DEHP can adversely impact lung development in

rats. The majority of the studies focusing on DEHP toxicity used the oral route of administration; however, the human sub-population with the highest exposure to DEHP is infants being treated in neonatal intensive care units, where exposure is mainly intravenous. Since DEHP is efficiently hydrolyzed in the gut to its active metabolite MEHP, the route of exposure can have a significant impact on the toxicological profile of DEHP. We investigated the effects of oral and intravenous (IV) administration of DEHP in the lungs of a neonatal male rat model. Following a modification of a study design by Cammack et al. (2003), five-day-old male Sprague-Dawley rats were dosed either by oral gavage or tail vein injection with 0, 60, 300, or 600 mg DEHP/kg bw/day for 5 or 21 consecutive days. Body weights were reduced relative to control in pups dosed for 21 days with 600 mg/kg/d DEHP, independent of the route of administration. Lung weights were not changed by any treatment. Five and 21 doses of IV, but not oral, 600 mg/kg/d DEHP caused lung interstitial inflammation and hemorrhage. Multifocal granuloma centered on capillary emboli were also observed in the alveoli of rats treated with IV, but not oral, DEHP in a dose-responsive manner, with a LOAEL of 300 mg/kg/d for 5 doses and 60 mg/kg bw/d for 21 doses. No effects were observed in testis of pups exposed to IV DEHP below 300 mg/kg bw/d for 5 or 21 doses. These data suggest that IV DEHP is more toxic to the lung than an equivalent oral dose, and that the lung may be more sensitive to IV DEHP than the testis in the neonatal rat model. Funded in part by NTP IAG 224-07-0007 between the FDA/NCTR and the NIEHS/NTP.

PS 618 JUVENILE TOXICITY ASSESSMENT OF ANIDULAFUNGIN IN RATS.

G. Chmielewski[1], C. J. Bowman[1], S. L. Ripp[1], E. M. Lewis[2], C. M. Sawaryn[1] and D. M. Cross[3]. *[1]Pfizer Inc., Groton, CT, [2]Charles River Laboratories Preclinical Services, Horsham, PA and [3]Pfizer Inc., Sandwich, United Kingdom.*

Anidulafungin (a marketed, non-competitive inhibitor of (1,3)-β-D-glucan synthase) is being evaluated for pediatric treatment of fungal infections, including neonates. Juvenile toxicity studies with anidulafungin were conducted to support neonatal dosing in the clinic as well as to evaluate long-term safety considerations. The first study was a single dose pilot study administered on Postnatal Day (PND) 7 either intravenously (IV), subcutaneously, or intraperitoneally to Fisher rats to determine a route of administration with exposure most consistent to intravenous infusion used in the clinic and adult nonclinical studies (not technically feasible in young rats). Subcutaneous administration was determined the most appropriate route and was utilized in a subsequent repeat dose range-finding study from PND 4 to 28 at 0, 10, 30, or 60 mg/kg/day. Pups at ≥30 mg/kg/day had reduced body weights. In satellite animals, anidulafungin was detected in brain, bone, and heart following a single dose, while plasma exposure on PND 4 and 28 increased in proportion with dose. In the pivotal juvenile study, rat pups were treated from PND 4 to 62 (followed by a 5 week recovery period) at 0, 3, 10, or 30 mg/kg/day to evaluate potential liver toxicity (adult rat target organ) and possible permanent effects on neurological function due to uncertainties associated with the blood brain barrier development in neonates. Anidulafungin-related effects included slightly reduced body weights, increased liver weights, and mild decreases in red blood cell mass with increased reticulocyte counts. There was no liver pathology and in the post treatment phase there were no effects on neurological function and no lasting effects on body weight or hematology. In conclusion, the juvenile rat no-adverse-effect-level (NOAEL) was 30 mg/kg/day (exposures similar to adult rat NOAEL) suggesting that juvenile rats are not more sensitive than adult rats to anidulafungin and that there are no permanent changes to neurological function.

PS 619 ORGAN SYSTEM ASSESSMENTS IN JUVENILE DOG TOXICOLOGY STUDIES.

K. Robinson, H. Penton, S. Y. Smith and M. Adamo. *Toxicology, Charles River Preclinical Services, Montreal, QC, Canada.* Sponsor: M. Vézina.

For some juvenile toxicology studies the dog is considered the most appropriate species. To meet regulatory guidances/requests from US FDA and EMA there may be a need to study the development of organ systems, including nervous, cardiovascular, gastro-intestinal, pulmonary, renal, immune, skeletal (growth) and reproductive, that develop in the postnatal period. Techniques for assessment of the development of many of these organ systems are readily available along with historical control data. However there are limitations in assessing some endpoints in the juvenile dog. For cardiovascular testing collection of direct or indirect blood pressure and electrocardiographs (ECG) is feasible. Direct assessment of blood pressure (BP) however has only been performed in dogs from 4 months of age. Considerations for this method include the size of the transmitter to implant relative to animal size as well as the BP catheter and ECG lead length to accommodate for rapid growth. Jacketed external telemetry has been conducted from 2 months of age with data collection for at least 24 hours. Pre-weaning ECG and indirect blood pressure using a tail cuff can also be performed. Variability is reduced when using direct recording. A full battery of assessments of skeletal growth ranging from measurements of height and length to imaging (bone densitometry) techniques and histomorphometry can be applied. For other evaluations, such as of the reproduction, the physiology of the dog precludes the conduct of meaningful testing. Screens for several systems such as renal and gastrointestinal are available. In others, such as the nervous and immune systems, tests such as neurological screens and phenotyping, respectively, are available. Neurological tests of learning and memory and immune function testing with T-cell dependent antibody response (TDAR) are under development. In conclusion, screening for effects on most major organ systems is feasible being mindful that there are limitations for some assessments due to age, test availability in toxicology laboratories and historical control data.

PS 620 CHEMICAL INTOLERANCE, ATTENTION DEFICIT/HYPERACTIVE DISORDER AND AUTISM—A LINK?

L. P. Heilbrun, R. F. Palmer and C. S. Miller. *Family Community Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX.* Sponsor: G. Gould.

Background: Chemical intolerance—the inability to tolerate a wide variety of everyday chemical exposures (fragrances, auto exhaust, cleaning compounds, etc.)—has been associated in numerous studies with multi-system symptoms including: asthma, chronic fatigue, cognitive and memory problems, etc. The primary objective of this study was to investigate the possible association between chemical intolerance and Attention Deficit/ Hyperactivity Disorder (AD/HD) and Autism Spectrum Disorder (ASD). Methods: A case-control study was used to test the hypothesis that mothers of children with AD/HD or ASD would score higher on the Quick Environmental Exposure and Sensitivity Inventory (QEESI©)—a validated tool for diagnosing chemical intolerance—than mothers whose children do not have either ASD or AD/HD. Mothers with (n =183) and without (n= 146) AD/HD children, and mothers with (n=282) and without (n=72) ASD children participated in separate online surveys. Results: Compared to control mothers, case mothers had significantly higher mean scale scores for Chemical Intolerance ($p < .001$), Other Intolerances (food, drug, caffeine, alcohol etc.) ($p=.01$), and Symptoms ($p<.001$) on the QEESI. Conclusions: These preliminary findings suggest a possible association between chemical intolerance, AD/HD and ASD. Further, if this association is confirmed by additional studies, the QEESI© may be a useful screening tool to identify mothers at risk for having children with AD/HD and autism, and to reduce this risk through early education and intervention—i.e., removal of suspect exposures prior to and during pregnancy.

PS 621 ANALYSIS OF TRANSFER RATE OF CONGENERS OF PCBS AND OH-PCBS FROM MOTHER TO FETUS THROUGH PLACENTA IN HUMAN.

C. Mori[1, 2], E. Todaka[1, 2], Y. Matsuno[1, 2], T. Fujisaki[1], M. Shimoda[2], H. Nakaoka[1, 2] and M. Hanazato[2]. *[1]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan and [2]Center for Preventive Medical Science, Chiba University, Chiba, Japan.*

The purpose of this study was to obtain the basic data of transfer rate of congeners of PCBs and OH-PCBs from mother to fetus in humans using umbilical cord, cord blood and maternal blood. Ten sets of samples (umbilical cord, cord blood and maternal blood) were collected at deliveries in Chiba University Hospital, Japan. PCBs (10 groups of PCB congeners by their number of chlorines from mono to deca) and OH-PCBs (3 groups of OH-PCB congeners by their number of chlorines from 5 to 7) were measured by GC/MS. The trasfer rate of total PCBs from maternal blood to cord blood and to umbilical cord were 21% and 13%, respectively. The transfer rate of PCB congeners (from Tri- to Deca-PCB) from maternal blood to cord blood were 29%, 23%, 22%, 20%, 18%, 16%, 9%, and 8%, respectively. The transfer ratio of PCB congeners (from Tri- to Deca-PCB) from maternal blood to umbilical cord were 19%, 16%, 15%, 13%, 11%, 9%, 3% and 4%, respectively. The transfer ratio of OH-PCB congeners (from Penta- to Hepta-PCB) from maternal blood to cord blood were21%, 20% and 18%, respectively. The transfer ratio of OH-PCB congeners (from Penta- to Hepta-PCB) from maternal blood to umbilical cord were15%, 13% and 11%, respectively. Our results showed that 1) the transfer ratio of each congenr was different, and 2) the transfer ratio ofhigher clorinated congenerswas smaller than that of lower clorinated congenersregarding both

PCB and OH-PCB. To evaluate the health effect on the next generation, it is necessary to estimate the contamination level of PCBs and OH-PCBs according to the congeners.

PS 622 ENDOCRINE EFFECTS OF A NOVEL HIV-NNRTI IN SEXUALLY IMMATURE FEMALE CYNOMOLGUS MONKEYS.

F. van Velsen[4], J. Sternberg[1], B. Niggemann[1], S. Friderichs-Gromoll[1], G. Weinbauer[1], I. Vanwelkenhuysen[2], L. Grooten[2], S. Lachau-Durand[4], S. De Jonghe[2], L. De Schaepdrijver[2], T. Coogan[3], L. Lammens[2], A. Raoof[4] and W. Coussement[2]. *[1]Covance Laboratories GmbH, Muenster, Germany, [2]GPCD, Janssen Pharmaceutica, Beerse, Belgium, [3]PCD, Centocor, Radnor, PA and [4]PCD, Tibotec, Beerse, Belgium.*

The compound has shown endocrine effects on adrenal glands and ovaries in rats and dogs indicative of adrenal CYP21 inhibition and early onset of ovulation (dogs). Non-human primates share with man a significant adrenal androgenic pathway. Inhibition of CYP21 may exert masculinization in prepubertal individuals as has been described for congenital adrenal hypertrophy due to CYP21 deficiency. The chosen model shares with women an identical physiology in terms of onset of puberty and ovulation. The endocrine effects were further evaluated in 1-2 year old female cynomolgus (Macaca fascicularis) treated for 8 weeks with 0, 200 and 500 mg/kg/day. The results on baseline and ACTH-stimulated hormone levels and standard toxicity parameters indicate that the NNRTI inhibits CYP21 also in the cynomolgus monkey. In addition, the compounds appeares to inhibit 17α-hydroxylase and 17,20 lyase (CYP17), key enzymes for the adrenal androgenic pathway. The enzyme inhibitions appeared to be partial and transient. The inhibition of CYP17 and the partial and transient inhibition of CYP21 alleviate the concern of masculinization of prepubertal children. The absence of any indication of early onset of puberty in the immature cynomolgus monkey model being a more relevant model for man neutralizes the concern due to the results of the dog studies. The lower dose tested in the cynomolgus monkeys was not without adrenal endocrine effects whereas the exposure to the compound was not significantly higher than that in adult patients treated with the clinical efficacious daily dose of 25 mg. Long-term clinical safety studies with higher doses than 25 mg qd have never resulted in any clinically relevant endocrine effect. Therefore, it is concluded that the immature cynomolgus monkey model is oversensitive to the adrenal endocrine effects of the NNRTI.

PS 623 IMPAIRED REPRODUCTION IN ADULT MALE, BUT NOT FEMALE, RATS FOLLOWING JUVENILE TREATMENT WITH THE AROMATASE INHIBITOR, EXEMESTANE.

G. Cappon[1], M. E. Hurtt[1], R. E. Chapin[1] and L. Burns-Naas[2]. *[1]DSRD, Pfizer, Groton, CT and [2]DSRD, Pfizer, LaJolla, CA.*

Aromatase inhibitors are currently being investigated in combination with growth hormone for the ability to improve final height in children. Exemestane is an irreversible steroidal aromatase inhibitor, structurally related to androstenedione. Because of its mechanism of action, the drug was investigated for the potential to affect mature reproductive systems when administered to immature rats. Male and female rat pups were treated with exemestane (0, 30, 100, 300 mg/kg) once daily from PND 7-50 (male) or 7-41 (female). Maturation of the reproductive system was evaluated by monitoring the onset of vaginal patency or preputial separation. After maturation, treated rats were mated with untreated rats to evaluate the potential impact on reproductive function. After mating (males) or on GD14 (females) rats were euthanized and reproductive organs evaluated microscopically. Exemestane was well-tolerated at all dose levels and there were no effects on age at onset of vaginal patency or preputial separation. There was no drug-related effect on female reproductive function. Treatment of juvenile male rats with exemestane caused an increase in cohabitation time prior to copulation and decreased copulation rates; however, pregnancy rates and litter size was not affected in rats that successfully mated. Decreased weight of the testis (10-15%) and epididymis (20-30%) were noted at al dose levels and the number of Sertoli cells was decreased in all dose groups. These data show that an aromatase inhibitor can reduce Sertoli cell proliferation during maturation and at clinically-relevant concentrations. The sensitive window for this effect is limited to the period of Sertoli cell proliferation, which is completed in rats by around PND 15 and prior to puberty in humans. Treatment beginning at a later time relative to the window for Sertoli cell proliferation or for a longer duration is not expected to have any additional adverse effect since the effect is not degenerative.

PS 624 MOST BPA-FREE PLASTICS RELEASE CHEMICALS HAVING ESTROGENIC ACTIVITY (EA): BPA-FREE DOES NOT MEAN EA-FREE.

G. Bittner[1, 2, 3], C. Z. Yang[1], S. Yaniger[2] and D. Klein[2]. *[1]CertiChem, Inc., Ausin, TX, [2]PlastiPure, Inc., Austin, TX and [3]University of Texas, Austin, TX.* Sponsor: R. Tice.

Scientists have recently expressed serious concerns about possible adverse health effects of bisphenol-A (BPA) that has estrogenic activity (EA) that causes many adverse health effects at low (pM-nM) doses, especially in fetal and juvenile mammals. We used a roboticized MCF-7 cell proliferation assay to quantify release of chemicals having EA in saline or ethanol extracts of many types of commercially available plastic materials. To properly detect release of chemicals having EA, we show that plastic products should be extracted with more-polar and less-polar solvents—and exposed to common-use stresses such as microwaving, UV radiation, and/or boiling. If only one extraction solvent is used and no common-use stresses are applied, then most plastic products are mischaracterized as EA-free. In contrast, when extracted with two solvent types and exposed to common use stresses, almost all (over 90%) commercially available plastic products, including those advertised as BPA-free, release chemicals having EA. As one example, all products tested made from Eastman's Tritan™ resins released chemicals having easily detectable EA—-often at levels greater than that released by BPA-based polycarbonate bottles. Products that do not release chemicals having EA can be cost-effectively made with currently available technologies by using chemicals with no detectable EA in all steps of the manufacturing process. Such EA-free products are producible at minimal additional cost. It is now realistically possible to eliminate the potential health risk posed by the leaching of chemicals having EA from plastics into food products. Supported by NIH SBIR44ES014806.

PS 625 MANGANESE LEVELS IN CARPET DUST ARE ASSOCIATED WITH PROXIMITY TO AGRICULTURAL USE OF MANEB AND MANCOZEB.

R. Gunier[1], D. R. Smith[2], A. Bradman[1] and B. Eskenazi[1]. *[1]University of California, Berkeley, CA and [2]University of California, Santa Cruz, CA.*

Rationale: Although manganese (Mn) is an essential nutrient, at high exposure levels Mn is a potent neurotoxicant. The fungicides maneb and mancozeb are ~21% Mn by weight and more than 140,000 kg of these pesticides are applied each year in the Salinas Valley, California. However, it is not clear whether these pesticides contribute to environmental Mn exposure to children. The objective of this study was to evaluate the relationship between Mn levels in carpet dust and residential proximity to reported agricultural uses of maneb and mancozeb. Procedures: We collected carpet dust samples from over 300 residences enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study. Carpet dust samples were sieved to < 150 μm, digested in 7.5 N nitric acid and analyzed using inductively coupled plasma optical emission spectroscopy. The latitude and longitude of each residence was determined using GPS. We used a geographic information system to summarize pesticide use for several distances around each residence (500, 1000, 1500 and 2000 m) and time periods prior to dust collection (7, 30, 90 and 180 days). We calculated Pearson correlation coefficients between Mn levels in carpet dust and the pounds of maneb and mancozeb applied for these combinations of distance and time. Results: We found detectable levels of Mn in all homes with a median concentration of 173 μg/g (range=8.4-414 μg/g; n=75 residences analyzed thus far). The strongest correlation (ρ=0.48) with Mn levels in carpet dust (μg Mn/g dust) was observed for the pounds of maneb and mancozeb applied within 1,500 m of the home during the 30 days prior to sample collection. Mn concentrations were also significantly higher in homes where farmworkers lived than in those without farmworkers ($p<0.01$). Conclusions: Mn concentrations in carpet dust were correlated with proximity to agricultural uses of maneb and mancozeb. Future analyses will incorporate additional residences, wind direction and more refined location of treated fields.

PS 626 BLOOD LEAD LEVELS AND IQ AMONG PRESCHOOL CHILDREN.

J. Liu[1], L. A. McCauley[2], J. Pinto-Martin[1], C. Yan[3], X. Shen[3] and H. Needleman[4]. *[1]University of Pennsylvania, Philadelphia, PA, [2]Emory University, Atlanta, GA, [3]Shanghai Jiaotong University, Shanghai, China and [4]University of Pittsburgh, Pittsburgh, PA.*

Background: Recent studies have shown that lead can cause intelligence deficits in children at blood lead levels(BLL) less than 10 ug/dL. This association has not been tested in China. Objective: To evaluate the association between BLL and IQ, especially at BLLs <10 ug/dL. Methods: The IQs of 1,344 preschool children enrolled

in the China Jintan Child Cohort Study was measured using Wechsler Intelligence Scale for Children (WISC). A questionnaire containing questions on covariates was completed. Children were categorized into three groups according to their BLLs (<5ug/dL, 5-10 ug/dL, and >=10 ug/dL). Performance IQ, verbal IQ, and full scale IQ were compared among the three groups. Linear regression models were fitted for boys and girls separately, adjusting for parental education, children's age, and iron intake. Results: Compared with boys with BLL<5ug/dL, boys with BLL>=10ug/dL were 3 points lower on performance IQ. Girls with the highest BLL had a 6 point deficit in performance IQ, and a 3 point reduction in verbal IQ compared to those with the lowest BLL. There were no significant differences between <5ug/dL and 5-10 ug/dL groups. In multivariate regression analysis, neither performance nor verbal IQ was associated with BLLs among boys. Among girls, a 1ug/dL increase in BLL was associated with a reduction in performance IQ of 0.5 points (p=0.048) and a reduction in verbal IQ of 0.5 points (p=0.074). However, when the analysis was limited to children with BLLs under 10 ug/dL, these associations were no longer significant. Conclusions: The present study suggest that lead can lower intelligence when BLL exceed 10 ug/dL, but may not do so at lower subclinical levels.

PS 627 CHARACTERISTICS OF HISTOPATHOLOGICAL CHANGES INDUCED BY SEMICARBAZIDE HYDROCHLORIDE IN RATS.

M. Takahashi, M. Yoshida, K. Inoue, T. Morikawa and A. Nishikawa. *Pathology, National Institute of Health Sciences, Tokyo, Japan.*

Semicarbazide (SEM), a metabolite of the banned veterinary antibiotic nitrofurazone, has recently been found in food in glass jars sealed with plastic gaskets using azodicarbonamide as a blowing agent, such as baby foods, jams and conserves. Although SEM is known to act as an osteolathyrogen, data for chronic toxicity remain insufficient. In addition, intake of SEM for infants is estimated to be much higher than for adults. Therefore, we performed (1) a 90-day toxicity study of SEM hydrochloride (SEM-HCl) using rats and (2) comparison of osteochondral and vascular lesions in young and adult female rats given SEM-HCl for 4 weeks. [Materials and methods] (1) Male and female Wistar Hannover GALAS rats fed diet containing SEM-HCl at 0, 250, 500 and 1,000 ppm for 90 days. (2) 3- and 20-week-old female SD rats were fed diet containing SEM-HCl at 0, 500 or 1,000 ppm, and 0 or 1,000 ppm, respectively, for 4 weeks. Half of the animals were then maintained for a further 2 weeks as recovery groups. [Results] (1) Deformations of the knee joints as well as thorax and tail were obvious from 500 ppm in both sexes. Histopathologically, disarrangement of chondrocytes and fissures in the cartilage matrix were apparent in epiphyseal and articular cartilage at all doses with dose dependency. In the thoracic aorta, appearance of elastic laminae was altered at all doses. (2) Young rats exhibited overt osteochondral lesions, such as disarrangement and thickening of epiphyseal cartilage and deformation of articular cartilage, but the severity of these lesions became reduced during the recovery period. In adult rats, although osteochondral lesions were relatively mild, reduction of severity was not obvious in the recovery group. In the thoracic aorta, appearance of elastic laminae was altered only in young rats in both the 4-week treatment and recovery groups. These results suggest that toxicological effects of subchronic exposure to SEM-HCI were mainly observed in bone, cartilage and the aorta, and growing animals have high susceptibility to SEM-HCl toxicity than adults.

PS 628 EFFECTS OF BLOOD LEAD LEVEL ON COGNITIVE AND PERSONAL ABILITY IN SCHOOL CHILDREN.

D. Kim[1], S. Yu[2] and J. Cha[3]. [1]*Department Environmental Health, National Institute of Environmental Research, Inchon, Inchon, Republic of Korea,* [2]*Department Environmental Health, National Institute of Environmental Research, Inchon, Inchon, Republic of Korea and* [3]*Lab. . Industrial Environment, Research Institute of Industrial health and Environment, Korean Industrial Health Association, Seoul, Seoul, Republic of Korea.* Sponsor: J. Park.

In order to evaluate the effects of lead concentration in the blood on cognitive and personal ability in school children in Korea, this study was designed to survey in three different types of areas, urban, fishery, and agricultural area, Korea. The Korean Intelligence Test-Primary was administered to the 302 school age children (154 3rd- grade and 155 6th- grade) in the three areas. Blood lead (Pb-B) was measured by means of atomic absorption spectroscopy equipped with graphite furnace atomizer as a cross sectional study.
Geometric means of Pb-B for boys and girls were 2.79±1.58 ug/dL and 2.54±1.51 ug/dL, respectively. No children exceeded Pb-B of 10 ug/dL that is the recommended level of Pb-B in children by CDC, U.S.A. When the subjects were divided by median into two groups, upper and lower Pb-B groups, the intelligence quotient(IQ) in cognitive ability of upper and lower groups were 106.4±13.7 and 110.0±14.9 respectively. IQ of upper group was low by 4 points than that of lower group. Hyperactivity and autism in personal ability showed different value significantly. These results were coincident with the large-scale study results in developed countries that chronic exposure to lead may reduce the intelligence of children. This study may provide the first suggestive evidence that Pb-B has an impact on childhood intelligence, hyperactivity and autism in Korean children. It is necessary to consider the effects of other important factors, such as parental intelligence in the next study.

PS 629 NEUROMOTOR AND SENSORY CHANGES IN ITALIAN CHILDREN RESIDENT IN THE VICINITY OF FERROALLOY INDUSTRY.

R. Lucchini[2, 1], F. Donna[2], S. Zoni[2], S. Guazzetti[3], E. Bontempi[2], D. R. Smith[1] and N. Zimmerman[4]. [1]*University of California, Santa Cruz, CA,* [2]*University of Brescia, Brescia, Italy,* [3]*PHS, Reggio Emilia, Italy and* [4]*Purdue University, West Lafayette, IN.*

Background and Objective: Increased parkinsonism was observed in Valcamonica, a valley in the province of Brescia, Italy. Prevalence data were higher in the vicinities of ferroalloy plants that emitted manganese (Mn), lead (Pb), iron (Fe), copper (Cu) zinc (Zn), and chromium (Cr) into the local environs. The aim of this study was to assess motor and neurosensory functions in adolescents in the exposed area and in a reference area. Methods: Adolescents were recruited for neurobehavioral examination. Metals were measured in airborne particles collected with 24-hour personal samplers, tap water and soil at the participants' homes. Biomarkers were collected for metal analysis including blood, urine, and hair. Independent variables considered as potential confounders/modifiers included gender, age, socio-economic status, parental education, and smoking and drinking habits. Results: A total of 311 children resident in both the exposed and the reference areas participated. Airborne Mn was 49.3 ± 5.6 ng/m3 (GM 29.0) in the exposed Valcamonica area and 27.3 ± 1.9 ng/m3 (GM 23.1) in the reference Garda Lake area. Similarly, there were significantly higher soil metal levels in the Valcamonica versus the Garda Lake area: Mn (median 986 ppm vs 416 ppm), Pb (median 46.1 ppm vs. 30.2 ppm); Fe (median 19800 ppm vs 13100 ppm). Mn in blood and urine were not significantly different between the two areas, whereas Mn in hair was significantly higher in subjects from the Valcamonica (GM 0.103 ppm) compared to the Garda Lake (GM 0.078 ppm). Multivariate analyses showed that children in the exposed area exhibited impairment of motor coordination and odor identification associated with airborne and soil Mn levels, while hair Mn was associated with altered odor identification. Conclusion: Environmental exposure to Mn in adolescents is related to deficit in motor and olfactory functions.

PS 630 JUVENILE (IMMUNO) DEVELOPMENT STUDY IN GÖTTINGEN MINIPIGS®.

A. Wolterbeek, G. van Mierlo, C. Snel, F. Kuper and A. Penninks. *Toxicology and Applied Pharmacology, TNO Quality of Life, Zeist, Netherlands.* Sponsor: R. Woutersen.

In the growing field of juvenile safety testing, the rat is the first species of choice but for translational and/or other scientific reasons the rat may not always be the appropriate species. For those cases, the minipig can be a good alternative but then the basic knowledge about the development of main organ systems needs to be extended. The aim of the present study was to gain more knowledge about 1) physical and sensory development of the minipig (developmental landmarks), 2) haematological and clinical chemistry parameters during development, 3) immunological parameters during development, 4) histology of main organs during development. Hereto, the physical and sensory development of the piglets of 5 sows was followed starting at birth. At an age of 2, 7, 13, 27 days and 2, 3 and 6 months blood was collected from 4 male and 4 female piglets for hematology, clinical chemistry and immunological parameters (lymphocyte subset analysis and natural killer (NK) cell activity); a series of organs and tissues was collected to study age-related and developmental histology and background histopathology. Physical and sensory parameters (e.g. eyes open, hair, teeth) showed that the minipig is relatively mature at birth. Quantitative and qualitative (functional) immunological endpoints were overall successfully implemented in these minipigs. Histopathology showed that some organs were (almost) fully developed at birth, while others showed distinct postnatal development (e.g. lymphoid organs such as thymus, bone marrow, spleen, lymph nodes, Peyer's patches, bronchus associated lymphoid tissues as well as other organs and tissues like pancreas, liver, adrenals, kidneys, male and female reproductive system, skin and brain). All data are being evaluated and compared to other species, including men, in order to improve the usefulness of the minipig in translational toxicology.

PS 631 NOVEL *DUOX* INDUCTION IN EPIDERMAL KERATINOCYTES BY IFN-γ AND IL-4.

T. Hill and R. H. Rice. *Environmental Toxicology, University of California at Davis, Davis, CA.*

Dual oxidase (DUOX) 1 and 2 are peroxide-generating members of the NOX family of membrane proteins. DUOX proteins have been implicated as peroxide donors during wound healing in mammalian mucosal epithelia and in hydroxylation events that lead to host defense barrier formation in plant tissues. Keratinocyte differentiation in mammals leads to formation of a moisture retaining, protective barrier to the environment. Our studies found DUOX was upregulated during keratinocyte differentiation, suggesting it may be an essential part of this process as well. We have previously shown that IL-4 and IFN-γ cytokine balance regulates DUOX mRNA expression in human airway epithelium *in vitro* in a dose-dependent manner, and that the IFN-γ signaling mechanism is independent of the canonical pathways. We consider this novel role for DUOX in keratinocyte differentiation in the context of response to Th1 and Th2 cytokine agonists. To determine how *DUOX* gene induction in keratinocytes is affected by Th1/Th2 cytokines, the responses of *DUOX1* and *DUOX2* to IL-4 and IFN-γ were examined in epidermal keratinocyte cultures using real time QPCR. Specific dose-response relationships were observed. Exposure to IFN-γ caused a 3 to 5-fold induction of *DUOX2* (EC50 50 ng/mL); no induction of *DUOX1* was observed. However, in contrast to our studies in HBE1 cells, exposure to IL-4 generated both a 3-fold induction of *DUOX1* and a 4 to 6-fold induction of *DUOX2* (EC50 of 10 ng/mL). These inductions were dependent on *de novo* protein synthesis, and no receptor crosstalk was observed between IL-4 and IFN-γ in these cells. Our data are biologically consistent with historically proposed DUOX functions and suggest that imbalanced Th1/Th2 cytokine expression might disrupt differentiation. These findings may help explicate anomalies in wound healing under physiologic, pharmacologic or environmental conditions that also alter cytokine profile. These and future studies to explicate the role of DUOX in keratinocytes may provide a novel mechanistic perspective to assess environmental and industrial toxicant exposure in damaged or healthy skin.

PS 632 *IN VIVO* DERMAL UPTAKE PATTERN FOR 20 DILUTE AQUEOUS ORGANIC CHEMICALS IS POORLY PREDICTED BY MODELS BASED ON *IN VITRO* DATA.

K. T. Bogen. *Health Sciences, Exponent, Inc., Oakland, CA.*

Updating a similar analysis done in 1994 that addressed only 9 chemicals, previously published data on *in vivo* dermal uptakes from dilute aqueous solution were expressed as permeability coefficients (Kp, in cm/h) for 22 organic chemicals (including 5 phthalates) containing C, H, O, N, S, and/or Cl, and for the organophosphate malathion. Multiple regression on physico-chemical properties yielded a highly predictive model, $\log Kp = 1.942 - 2.297/\sqrt{(\log Kow)} - 0.007551\ MW$ ($r = 0.967$), for 20 of the 22 considered chemicals ($\log Kow \geq 0.8$), where log = base-10 logarithm, Kow = octanol:water partition coefficient, and MW = molecular weight. Butylparaben (a cytotoxic antimicrobial) and malathion (the sole organophosphate considered) were excluded as outliers, with Kp values ~10-fold less and ~100-fold greater than predicted, respectively. While *in vivo* Kp measures are all predicted within 2-fold for 20 chemicals, geometric mean errors >10-fold are predicted by five previous models fit only or primarily to *in vitro* diffusion-cell data. Those earlier models include two (one ~20 years old still used by USEPA) intended to adjust estimates of steady-state diffusion measured *in vitro* to better predict total uptake from dilute aqueous solution when non-steady-state exposure conditions may also occur. Models fit solely or primarily to measures of aqueous organic chemical penetration through dermal tissue *in vitro* thus cannot yet reliably predict corresponding dermal uptake measured *in vivo*. Consequently, regulatory approaches to dermal exposure assessment that rely primarily or exclusively on *in vitro* data remain seriously flawed. Instead, models fit only to *in vivo* data should be preferred for non-hydrophilic chemicals, and more *in vivo* data should be generated to support improved, accurate dermal exposure assessment for such aqueous organics, until wide discrepancies between dermal uptakes measured *in vivo* and those measured *in vitro* can be explained and predicted reliably.

PS 633 PREDICTING SKIN PERMEABILITY FROM COMPLEX CHEMICAL MIXTURES: THE IMPACT OF BIOLOGICAL SKIN MODEL SYSTEMS ON QUANTITATIVE STRUCTURE PERMEATION RELATIONSHIPS (QSPR).

J. E. Riviere and J. D. Brooks. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Dermal absorption of topically applied chemicals usually occurs from complex chemical mixtures, yet most attempts to predict dermal permeability use data collected from aqueous binary mixtures. The focus of this research was to develop quantitative structure permeation relationships (QSPR) for predicting chemical absorption from mixtures through skin using two different *in vitro* porcine skin biological systems. A total of 16 diverse chemicals were applied in 384 treatment combinations in pig skin flow-through diffusion cells (PSFT) and 20 chemicals in 119 treatment combinations in the isolated perfused porcine skin flap (IPPSF). Apparent permeability coefficient (k_p*) was calculated using chemical flux into perfusate in the PSFT, while area under the curve (AUC) was calculated for the IPPSF. These data were then fit with a modified Abraham and Martin dermal QSPR model including a sixth term called a mixture factor (MF) to account for mixture interactions based on physico-chemical properties of the mixture components. Goodness of fit was assessed using correlation coefficients (r^2), internal and external validation metrics (q^2_{LOO}, $q^2_{L25\%}$, q^2_{EXT}), and applicable chemical domain. Different mixture factors were needed for each model system. Based on standard deviation and p-values of the model parameters greater than α=0.05, the Abraham and Martin model could be reduced to four terms plus the MF for each biological system. The term which could be eliminated from each model differed in the PSFT (hydrogen-bond acceptor basicity) and in the IPPSF (dipolarity/polarizability). These findings suggest that a QSPR model for estimating apparent k_p* as a function of chemical mixture composition is possible and that the nature of the QSPR model selected is dependent upon the biological level of the *in vitro* test system used. Both of these findings having implications when dermal absorption data is used for *in vivo* risk assessments. (Supported by NIOSH OH-07555)

PS 634 *IN VITRO* EVALUATION OF THE EFFECT OF DOSE, RECEPTOR FLUID, AND VEHICLE ON THE ABSORPTION OF FIVE PHYSICOCHEMICALLY DIFFERENT COMPOUNDS IN PORCINE SKIN.

D. Karadzovska, E. L. Koivisto, J. D. Brooks and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Understanding the interactions that control the extent and rate of passive drug absorption through skin is of great interest in pharmaceutical and toxicological research. These interactions involve the physicochemical properties of the test compound, the vehicle, and the anatomy of the skin. This research aimed to identify the rate limiting factors which determine the extent and rate of compound absorption through porcine skin from three vehicles (propylene glycol, water and ethanol), by testing the effect of common experimental variables. Two traditional *in vitro* diffusion cell systems (static and flow-through) were employed to evaluate the effect of finite (20 μL) and infinite (1000 μL) doses and the presence/absence of bovine serum albumin (BSA, 4.5%) in the receptor. Five compounds (caffeine, diclofenac sodium, mannitol, salicylic acid and testosterone) selected on the basis of lipophilicity, molecular weight and commercial 14-C availability were applied at a concentration of 1.28 μg/μL. Flux of each compound into the receptor phase was monitored over 24 hours. Levels of radioactivity were also determined in the stratum corneum (assessed by tape stripping) and the remaining epidermis. Apparent permeability coefficients and percentage of the applied dose absorbed were then calculated and compared. Each compound had its own unique absorption profile. A slight variation in the percent dose absorbed was observed when comparing the presence/absence of BSA, being more evident with the finite doses. The correlation between absorption data from both diffusion cell systems was stronger when BSA was present. For the majority of the compounds, absorption was greatest with a water vehicle. For the infinite dose, the dominant absorption pattern was water>ethanol>propylene glycol. This suggests the influence of these variables should be considered when designing predictive absorption models. (Supported by Novartis Animal Health, Inc.)

PS 635 CHARACTERIZATION OF NORMAL SKIN THICKNESS FOR VARIOUS BODY REGIONS, AGES, AND GENDERS OF YUCATAN MINIATURE SWINE.

L. Brown[1], D. Kim[2], C. Hanks[1], D. Brocksmith[1], M. Hodges[1], J. Liu[1] and G. Bouchard[1]. [1]*Sinclair Research Center, Columbia, MO and* [2]*VMDL CVM University of Missouri, Columbia, MO.*

This poster presents the results of a histologic skin characterization study in the Yucatan minipig. Four skin samples [neck, back (lumbar), flank, abdomen] were collected by 8-mm punch biopsy from various age animals and immediately fixed in 10% neutral buffered formalin. The number of hairs per surface area were enumerated then samples were processed, embedded in paraffin, sectioned, and stained with H & E for histologic evaluation. The strateum corneum, cellular epidermis, and dermis layers were measured microscopically using an image analyzing system. Each component measurement was taken at random 5 times and averaged for each sample. Gender, age and body region specific means (μM), ± SDs, and observed ranges are presented for 5 inter-follicular skin components (full thickness, stratum

corneum thickness, cellular epidermis thickness, number epidermal cell layers, and dermis thickness). Relative ratios of epidermis and dermis to full thickness measurements were calculated. The differences in thickness for various Yucatan body sites is predominantly due to differences in dermis thickness as the dermis makes up 92-98% of full-thickness skin. Rump skin was thinner than back skin for the two samples analyzed. Female skin was thinner than male skin. Abdomen skin was generally thinner than the other sites evaluated. The cellular epidermis number of cell layers varied from 3-7 layers (mean 4.64 layers) and the trichogram from 0-39 hairs (mean 5.27 hairs) counted per 50.24 sq mm. Data were compared to published measurements of adult human skin illustrating similarities or differences. These data support the premise that young adult Yucatan minipig skin thickness is similar to human skin thickness. These cutaneous measurement data will aid investigators contemplating a dermal study utilizing the Yucatan miniature swine model.

PS 636 NORMAL PHYSIOLOGICAL RANGES FOR HANFORD MINIATURE SWINE.

M. Ross, L. Brown, D. Unterreiner, C. Hanks, J. Liu, M. Hodges and G. Bouchard. *Sinclair Research Center, Columbia, MO.*

The miniature swine have been increasingly recognized as a non-rodent model in regulatory toxicity. Members of the FDA have even published on the use of miniature swine as an alternative to canine and non-human primates in regulatory toxicity. The similarities between the cardiovascular, renal, and digestive systems make the miniature swine a suitable animal to model the human counterpart. The miniature swine are also the most recognized species for dermal toxicology. The Hanford miniature swine (HMS) has other attractive traits that make them a good substitute to model humans. They are omnivorous, easy to handle, prone to obesity, and will develop atherosclerosis and dyslipidemia if fed a high fat diet. With the advent of new techniques, all routes of compound administration can be used with miniature swine. The HMS should be considered as one of the non-rodent species in toxicity testing. In an effort to generate a database on baseline information about the normal physiological status of the Hanford miniature swine, we report expanded physiological data from normal intact and naïve juvenile and young adult miniature swine of both genders. The normal physiological data gathered includes growth parameters, hematology, serum chemistry, coagulation profile, urinalysis, ECG rhythm and segment intervals, and organ weights.

PS 637 ROLE OF MAP KINASES IN REGULATING EXPRESSION OF KERATINOCYTE ANTIOXIDANTS BY THE ELECTROPHILIC NITRO-FATTY ACIDS DERIVATIVES 9- AND 10-NITROOLEIC ACID.

R. Zheng[1], A. T. Black[1], A. Gow[1], D. E. Heck[3], D. L. Laskin[1] and J. D. Laskin[2]. [1]*Rutgers University, Piscataway, NJ,* [2]*UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ and* [3]*New York Medical College, Valhalla, NY.*

ABSTRACT BODY: In many tissues, chemical-induced injury results in the production of the reactive second messenger nitric oxide. It is well recognized that nitric oxide and a variety of its byproducts including nitrite can contribute to tissue damage and form novel signaling mediators via redox mediated nitration reactions. Nitration of unsaturated fatty acids can generate electrophilic nitro-fatty acid derivatives such as 9-nitrooleate (9-NO) and 10-nitrooleate (10-NO), which are known to initiate cell signaling pathways. In the present studies we characterized nitro-fatty acid-induced signaling in PAM212 mouse keratinocytes. Treatment of the cells with 5-25 μM 9-NO or 10-NO for 6 hr was found to upregulate mRNA expression for the primary antioxidants catalase (3-6 fold) and heme oxygenase-1 (HO-1, 50-100 fold), and the secondary antioxidants glutathione-S-transferase (GST) A1/2 (40-80 fold), GSTA3 (10-70 fold), and GSTA4 (5-15 fold), as determined by real time PCR. 9-NO also induced phosphorylation of the JNK and p38 MAP kinases indicating that the nitro-fatty acid activated MAP kinase signaling. Treatment of keratinocytes with inhibitors of JNK (SP600125, 20 μM) or p38 (SB203580, 10 μM) MAP kinase markedly suppressed 9-NO and 10-NO-induced expression of heme oxygenase-1 and GSTA4, but not catalase, GSTA1-2 or GSTA3. These data indicate that the antioxidants are regulated by distinct mechanisms in the cells. Western blot analysis showed that 9- and 10-NO induced HO-1 protein, a response markedly reduced by inhibition of JNK, and to a lesser extent by p38 kinase. Taken together, these data indicate that nitro-fatty acids are important in the control of antioxidant expression in mouse keratinocytes. Moreover, MAP kinase signaling plays a key role, at least in part, in regulating responses to the nitro-fatty acids. Supported by CA132624, ES004738, ES005022, GM034310 and AR055073.

PS 638 A STUDY OF PHOTOTOXICITY FOLLOWING INTRAVENOUS ADMINISTRATION OF CIPROFLOXACIN HYDROCHLORIDE IN THE MICROMINIPIG.

H. Kawaguchi[1], N. Miyoshi[1], A. Tanimoto[1], Y. Takahashi[2], S. Utsunomiya[2], T. Motokado[2], Y. Ooshima[2], H. Izumi[2], T. Sukamoto[2] and R. Nagata[2]. [1]*Kagoshima University, Kagoshima, Japan and* [2]*Shin Nippon Biomedical Laboratories (SNBL), Ltd., Kagoshima, Japan.*

Purpose: We previously reported (2008 and 2009 SOT) on in vivo phototoxicity in Balb/c mice. Recently, the Microminipig (MMP; Fuji Micra Inc.) has emerged as a possible experimental animal model for non-clinical pharmacological/toxicological use. The body weight of the young mature MMP is less than 7 kg, enabling ease of handling. In this study, we evaluated phototoxicity in the MMP following intravenous administration of ciprofloxacin hydrochloride (CPFX) because little information is available on in vivo phototoxicity. Method: Five female MMPs aged 5-7 months were used. The backs of the animals were shaved, and divided into four areas on both the right and left sides before administration. The areas on the right side were not irradiated. CPFX (a phototoxic substance) was continuously administered intravenously once at 100 mg/kg. Approximately fifteen minutes after the start of administration, the four areas on the left side of each animal were irradiated with long-wave ultraviolet rays (UVA) at approximately 5, 10, 15, or 20 J/cm2 with an Ultraviolet Irradiation Apparatus (Dermaray, M-DMR-50, Eisai Co., Ltd.). Skin reactions (erythema and edema) were evaluated in accordance with the Draize method criteria before administration and at approximately 1, 4, 8, 12, 24, 48, and 72 hours after irradiation. After gross examination at each observation point, the irradiated sites were prepared for histopathology. Result: At approximately 1 hour after irradiation, no skin reaction (erythema and edema) was grossly observed at any irradiation site in any animal. Erythema scored as 1-3 was observed at all irradiated sites from 12 to 72 hours after irradiation. Edema scored as 1 was observed at the 15 and 20 J/cm2 UVA irradiation sites from 48 to 72 hours after irradiation. Positive reactions were clearly observed, from which it was considered possible to evaluate the in vivo phototoxicity test using the MMP.

PS 639 INFLUENCE OF THE ARYL HYDROCARBON RECEPTOR ON PROLIFERATION OF HUMAN KERATINOCYTES (HACAT).

M. Kalmes, J. Hennen, J. Clemens and B. Blömeke. *Environmental Toxicology, University of Trier, Trier, Germany.*

While activation of the aryl hydrocarbon receptor (AhR) by exogenous ligands is well investigated, its physiological function is less understood. By extending research in AhR biology, evidence appeared that the receptor generally plays an important role in cell physiology. In keratinocytes, little is known about endogenous functions of the AhR. In order to expand this knowledge, we analyzed the impact of AhR knockdown on cell cycle progression in HaCaT cells and showed that proliferation of siAhR HaCaT cells was significantly decreased. In line with that result, western blot analysis revealed that protein level of the cyclin dependent kinase inhibitor p27KIP1 was increased, whereas protein level of the cyclin dependent kinase (CDK) 2 was reduced. CDK4 and CDK6 protein levels remained unchanged, whereas protein level of the retinoblastoma protein (pRB) was reduced. By measuring ethoxyresorufin-O-deethylase (EROD) activity we showed that endogenous cytochrome P450 is required for normal cell cycle in HaCaT cells, as well. To the best of our knowledge we provide evidence for the first time in human skin cells that in the absence of exogenous ligands the AhR promotes cell cycle progression in HaCaT cells and one can speculate that this is the physiological function of this receptor in keratinocytes.

PS 640 WHAT IS THE BEST SKIN PREPARATION FOR DERMAL PENETRATION STUDIES *IN VITRO* ? A COMPARISON OF FULL-THICKNESS SKIN AND DERMATOMED SKIN.

K. Guth[1], M. Schaefer-Korting[2], E. Fabian[1], B. van Ravenzwaay[1] and R. Landsiedel[1]. [1]*BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany and* [2]*Freie Universitaet, Berlin, Germany.*

Skin Absorption in vitro is an alternative method accepted by the OECD. The respective guidelines (TG 428 and GD 28) give technical guidance how to perform valid experiments. Within these guidelines many experimental parameters can be varied, with potential influence on the study results. We investigated the influence of skin preparations on dermal penetration and measured dermal penetration with full-thickness skin (FTS) and dermatomed skin (DMS) from the same human

donor. The experiments were performed as finite dose studies with radio-labeled Testosterone, Caffeine, (4-Chloro-2-methylphenoxy)acetic acid (MCPA) and Mannitol in modified Franz type diffusion cells. 10 µl of the test substance preparation were applied on 1 square cm of the skin preparation. The exposure time was 6 h and after a first skin wash, the post observation period was 18 h. Penetrated test substances were quantified by liquid scintillation counting and were used to calculate the maximal penetration rate, the lag time and the permeability constant (Kp). Moreover the absorbed dose was calculated from the amount of test substance in the receptor fluid and the skin. Testosterone absorption was 20 ± 5 and 12 ± 1% for FTS and DMS, respectively. For Mannitol it was 27 ± 15 and 13 ± 7%, respectively and for MCPA 17 ± 3 and 12 ± 2%, respectively. For Caffeine the absorption was 47 ± 14% in both skin preparations. Generally, Kp was higher in DMS compared to FTS (e.g. for Testosterone 21x and 90x 10-5cm/h, respectively). Moreover, FTS had higher amounts of test substance remaining in the skin and often lower amounts in the receptor fluid compared to DMS and the lag time was longer. The absorbed doses were, however, assessed to be comparable for FTS and DMS within the variability of this study.

PS 641 ANTI-INFLAMMATORY DRUG EFFICACY FOR TREATING SULFUR MUSTARD INDUCED CUTANEOUS LESIONS.

J. L. Plahovinsak[1], M. D. Etheridge[1], J. Rhone[1], F. M. Reid[1], J. F. Dillman[2] and J. S. Graham[2]. *[1]Battelle, Columbus, OH and [2]U.S. Army Medical Research Institute of Chemical Defense, Aberdeen, MD.*

Battelle's Biomedical Research Center and the U.S. Army Medical Research Institute of Chemical Defense have aligned research efforts for efficacy testing of candidate drugs to promote wound healing of sulfur mustard (SM)-induced injuries. Using an established SM dermal weanling swine model, this GLP-compliant study evaluated the efficacy of a non-steroidal anti-inflammatory drug, diclofenac sodium with the steroid, clobetasol propionate. Sites on the ventral abdomen of each female Yorkshire-cross pig were exposed to 400 µL of neat SM. Two sites on one side of the ventral midline were superficial dermal injuries (SD, 8 min) and two sites on the opposite side were deep dermal injuries (DD, 30 min). Treatments were applied to one site per depth at 2 hours post-exposure, twice daily, for 5 days. Parameters measured on Study Days 0 (transepidermal water loss and infrared imagery), 2, 3, 7, and 14 included digital photos, wound size measurements, modified Draize Scoring, and non-invasive bioengineering methods (transepidermal water loss, reflectance colorimetry, and infrared imagery). Tissues were collected on Day 14 and processed for histopathology and immunohistochemistry. At 14 d, treatment of SD injuries with diclofenac sodium and clobetasol did not significantly influence healing, as the untreated sites were nearly healed. However, treatment of DD injuries enhanced healing as evidenced through increased re-epithelialization and epidermal hyperplasia. Decreased severity of dermal, follicular, sweat gland, and smooth muscle necrosis was observed in the treated DD lesions. Immunohistochemical localization of collagen type VII and CD49f (alpha 6) was observed in SD sites. Untreated SD sites showed decreased immunospecificity for CD49f compared to the treated sites. For DD injuries, equivalent immunospecificity for collagen type VII between treated and untreated sites was observed. This work was supported by the U.S. Army Medical Research and Materiel Command under Contract W81XWH-05-D-0001, Task Order 10.

PS 642 DERMAL ABSORPTION OF DICYCLOHEXYLAMINE (DCHA) IN METAL WORKING FLUID FORMULATIONS.

R. Baynes, A. Linthicum, J. Yeatts, J. Brooks and E. Koivisto. *North Carolina State University, Raleigh, NC.*

Dicyclohexylamine (DCHA) is commonly used in the metal working industry to prevent corrosion of fabricated materials. In the last decade there has been increased use of DCHA as a biocide in metal-working fluids (MWFs); although it is not registered by the U.S. EPA for this purpose. There is no published information describing the dermal absorption of DCHA in spite of the fact that machine workers are most likely to be exposed by the dermal route than any other route and DCHA is a known dermal irritant. The objective of this research was to quantify the dermal absorption of DCHA in vitro using pig skin because pig skin is similar to human skin anatomically and biochemically. DCHA was applied to pig skin in 3 generic MWF formulations commonly used in industry: soluble oil (SO), synthetic (SYN), and semi-synthetic (SS). Dermatomed pig skin (n = 5-6) was loaded in a flow-through diffusion cell system with the dermal surface perfused for an 8-hr exposure to mimic occupational exposures. Dosing solutions containing MWFs or water were prepared below or near the saturated solutions for DCHA to provide finite or infinite dosing conditions. There was an initial 1.0 hr lag period and peak fluxes occurring at 2.0 to 3.0 hrs for all of the formulations tested. Dermal absorption of DCHA was greatest from SS (12.5%), but significantly less from SO (4.6%) and SYN (5.9%) formulations. Surprisingly, 81% DCHA was absorbed from water mixtures. These preliminary results suggest that DCHA can more readily diffuse across pig skin from aqueous solutions than from complex MWF formulations. This may be due to differential partitioning behavior within the formulations. In conclusion, there are significant differences in absorption of DCHA across the various MWF formulations, thus it is imperative to consider such differences in dermal risk assessments involving this widely used additive in the metal machining industry. This work was supported by NIOSH grant R01-01-03669.

PS 643 INTERLEUKIN 6 EXPRESSION MODULATES IRRITANT DERMATITIS SEVERITY.

E. G. Lee, B. M. Mickle and R. Gallucci. *Pharmaceutical Sciences, Oklahoma State University Health Sciences Center College of Pharmacy, Oklahoma City, OK.*

Of reported occupational injury associated with workman's compensation, contact dermatitis ranks second most prevalent over all. Inflammatory cytokines are known to be closely involved in dermatitis, and modulation of various cytokines by specific irritants could exacerbate skin damage contributing to increased irritancy. If specific cytokines can be associated with irritancy, this may be of significant predictive value when judging the irritancy potential of a chemical, or the response of an individual to irritant exposure. Interestingly JP-8 jet fuel, which is non-corrosive yet causes severe irritant dermatitis, decreases the expression of the inflammatory cytokine IL-6 in exposed skin. Because IL-6 is paradoxically associated with both skin healing and inflammation, it may be that chemically induced, or genetically associated modulation of skin IL-6 levels contributes to severity of dermatitis. To investigate this, the skin of IL-6 deficient (KO), IL-6 over-expressing transgenic (Tg), and wild type (WT) mice was exposed to the irritants JP8 jet fuel, benzalkonium chloride, and acetone as a control. Skin samples were collected after up to 7 days of exposure and were assessed for inflammation by visualization of dermatoses, histopathology, and inflammatory cytokine expression. It was found that KO mice displayed significantly greater levels of inflammation as compared to WT and Tg mice. By 7 days, there is a nearly six-fold increase in inflammatory M1 macrophages in JP8 treated IL-6KO skin, while no increase was apparent in Tg skin. Similarly, the expression of the inflammatory cytokines IL-1, CCL3, CCL11, and CCL20 was increased 4-6 fold after seven days of JP8 treatment in KO skin, where very small changes were apparent in Tg skin. These data indicate that IL-6 is indeed not pro-inflammatory in skin, but rather is associated with resistance to irritancy. Thus, IL-6 could prove to be a useful marker in determining irritancy potential of a chemical or susceptibility of an individual to irritant dermatitis. Supported by NIOSH grant 5R03OH009662.

PS 644 THE EPIOCULAR™ ASSAY FOR TESTING EYE IRRITATION *IN VITRO* : IN HOUSE VALIDATION WITH 60 TEST SUBSTANCE IN A ROUTINE LAB OF THE CHEMICAL INDUSTRY.

A. Schrage[1], S. Kolle[1], B. Wareing[1], R. B. Tacou[1, 2], B. van Ravenzwaay[1], H. Kanderova[3] and R. Landsiedel[1]. *[1]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany, [2]Master Program in Toxicology, Technical University of Kaiserslautern, Kaiserslautern, Germany and [3]MatTek Corporation, Ashland, MA.*

The bovine corneal opacity and permeability (BCOP) test was regulatorily accepted for the identification of corrosive and severe ocular irritants (GHS category 1) in 2009. But no in vitro test has been regulatorily accepted for the differentiation of ocular irritants and non-irritants (GHS category 2). Human reconstructed tissue models have been suggested for incorporation in a tiered test strategy to ultimately replace the Draize eye irritation test (OECD405). These models use cell death as an indicator of ocular irritation. We established and evaluated the EpiOcular assay to discriminate irritants from non-irritants. Test substances that decreased viability to ≤ 60% (compared to control) are considered eye irritants (GHS cat 1 or cat 2) and test substances with less effect are considered non-irritants. In addition to the EpiOcular Test we performed the BCOP assay (OECD437) and the direct peptide reactivity assay (DPRA, Gerberick). The tests were performed with 60 test substances including a broad variety of chemicals and formulations for which in vivo data (Draize test) were available: 18 severe irritants/corrosives (GHS category 1), 21 irritants (GHS category 2), and 21 non irritants. For the assessed data set the EpiOcular assay had a sensitivity of 90%, a specificity of 73% and an overall accuracy of 81%. Applying a lower viability threshold (50% instead of 60%) resulted in 82, 82 and 82%, respectively. The DPRA assay was not useful for prediction of eye irritation potential of the 60 compounds. Whereas the BCOP assay was – as expected – useful in further differentiating strong irritation effects. We will use the EpiOcular assay and the BCOP assay in a testing strategy to identify strong eye irritation, eye irritation and no eye irritation effects of test substances in routine testing of industrial chemicals and agrochemical formulations.

PS 645 EVALUATION OF THE *IN VITRO* EPISKIN AND SKINETHIC RHE SKIN IRRITATION TEST METHODS FOR HAZARD IDENTIFICATION OF CHEMICALS.

D. Lelièvre[1], N. Alépée[1], J. Cotovio[1], M. H. Grandidier[1], A. De Brugerolle de Fraissinette[2], C. Tornier[2] and J. R. Meunier[1]. [1]*L'Oréal, Aulnay sous Bois, France and* [2]*SkinEthic Laboratories, Lyon, France.* Sponsor: H. Toutain.

On July22nd, 2010, the in vitro skin irritation OECD Test Guideline TG439 and the supportive Background Document were adopted. As reflected in the OECD TG439, the EpiSkin, designated as the Validated Reference Method and the SkinEthic RHE method can be considered to be applied to a wide range of physicochemical substances and mixtures. The evaluation of both the reproducibility and the reliability of these methods was performed to confirm their ability to predict the classification of chemicals in the former and the recent implemented Global Harmonization System (GHS) classifications applied in Europe. The present study purpose was to investigate the performances of both test methods with an expanded set of 62 chemicals in both classifications. Chemicals with in vivo scores between 2 to 2.3 are now considered as non-irritants. Consequently to the new Classification Labelling Packaging (CLP) system, the number of No category substances were increased from 37 to 45 whereas the irritant category number decreased to 17. When comparing the obtained results in the old versus the new European classification systems, the sensitivities of both, the in vitro Validated Reference EpiSkin method and the SkinEthic RHE test method were improved to 88.2 and 94.1%, respectively. Accuracies of both test methods were 83.9 and 77.4 respectively. Overall performances were in accordance with criteria defined in the Annex 2 of OECD TG439. These results confirm that these two test methods can be considered as reliable in vitro screening tools to assess the skin irritation potential of chemicals for the REACh registration and the European Cosmetic Directive legislation.

PS 646 ADAPTATION OF THE VALIDATED SKINETHIC RHE SKIN CORROSION TEST METHOD TO 0.5 CM2 TISSUE SAMPLE.

C. Tornier[1], M. Roquet[1], N. Alépée[2], J. R. Meunier[2] and A. De Brugerolle de Fraissinette[1]. [1]*SkinEthic Laboratories, Lyon, France and* [2]*L'Oréal, Aulnay sous Bois, France.* Sponsor: H. Toutain.

The Globally Harmonised System for the classification and labelling of chemical substances and mixtures (GHS) defined skin corrosion as the production of irreversible tissue damage in the skin. In vitro human reconstructed epidermal models have been used to develop protocols able to discriminate corrosives from non corrosives. The SkinEthic RHE test method, using 0.63 cm2 inserts, was validated by ESAC (ECVAM advisory board) in 2006. Due to manufacturing constraints, the SkinEthic RHE model is now produced on 0.5 cm2 instead of 0.63 cm2. The present study first aim to demonstrate that the RHE skin corrosion protocol could be adapted from 0.63 cm2 to 0.5 cm2 RHE tissues samples. For such purposes, the protocol size adaptation was performed using the 25 chemicals including the 12 OECD TG431 reference test substances. To test the robustness and relevance of the test method, particular attention was given to choose chemicals correctly classified (non corrosive, corrosive) but also misclassified chemicals (false positives / negatives). In addition, the objective was to show that the quality of RHE tissues was not only maintained but also improved. The quality control, performed on 136 (0.63 cm2) and 262 (0.5 cm2) RHE batches, showed a mean of 0.991 and 1.169, respectively. This similarity over the 9 years demonstrates the high quality production of the tissues using both viability and morphology parameters. Results obtained with the 0.5 cm2 skin corrosion test method showed that all corrosives (12) were correctly classified, and 11 out of 13 chemicals were identified as non corrosives. The 2 misclassified chemicals were also identified as false positives in the reference validated EpiSkin test method. The overall accuracy over the 25 chemicals was 92%. The specificity and the sensitivity of the 12 OECD TG431 reference chemicals were 100%. In conclusion, the quality of 0.5 cm2 SkinEthic RHE tissues was thoroughly maintained over 9 years and the performance of the skin corrosion test method fully meet the OECD and ECVAM requirements.

PS 647 EXPRESSION AND INDUCTION OF XENOBIOTIC METABOLISM GENES IN THE STRATATEST® HUMAN SKIN MODEL.

C. Rasmussen, K. Gratz, N. Simon, M. VanderZanden, C. Johnston and L. Allen-Hoffmann. *Stratatech Corporation, Madison, WI.*

Human *in vitro* skin models have shown broad utility for toxicological testing due to the similarity to the *in vivo* state and the ability to evaluate compounds as dosed in actual use or exposure. Importantly like human skin, which is metabolically competent, numerous studies have confirmed the expression and induction of phase I and phase II metabolic enzymes in keratinocytes and skin models. In the present study, the metabolic competency of the StrataTest® human skin model was investigated by evaluating the expression and induction of genes involved in xenobiotic metabolism. The StrataTest® full-thickness human skin model, containing both epidermal and dermal components, faithfully recapitulates many of the biological characteristics of human skin. The model is generated using NIKS® keratinocytes, a clinically-tested and consistent source of non-tumorigenic, pathogen-free human keratinocyte progenitors. To confirm that NIKS® keratinocytes provide appropriate metabolic capacity, gene expression profiles of phase I and phase II enzymes from NIKS® and primary keratinocytes were compared. Gene expression profiling was performed. Differences in expression of two-fold or greater between NIKS® and primary keratinocytes were considered significant. Concordance for expression of 51 phase I and phase II metabolic enzymes in NIKS® and primary human keratinocytes was 98%. RT-PCR and qPCR were employed to evaluate baseline gene expression and induction after exposure of the skin model to xenobiotic agents. Cytochrome p450 1A1 (CYP1A1) and 1B1 (CYP1B1) were weakly expressed constitutively, while N-acetyltransferase 1 displayed more robust constitutive expression. Upon exposure to 3-methylcholanthrene, CYP1A1 and CYP1B1 expression was strongly induced. Induction of CYP1A1 was also confirmed in skin tissues exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. These results show that similar to other *in vitro* skin models, StrataTest® skin tissues express genes critical to xenobiotic metabolism, further demonstrating the utility of this model for toxicological testing applications.

PS 648 INTRAMUSCULAR INJECTION SITES: IS THE QUALITY OF THE DIAGNOSIS IMPROVED WHEN MORE THAN ONE TISSUE SECTION IS EXAMINED?

C. Thuilliez, M. Perron-Lepage, C. Clément, J. Briffaux and C. Botteron. *Ricerca Biosciences SAS, Lyon, France.*

Microscopic evaluation of intramuscular injection sites at our Laboratory involves the examination of three tissue pieces from each site, in order to maximize the chance of examining the area where the injected material was deposited. We retrospectively studied the extent to which the quality of the diagnosis is maintained if only one section per injection site is examined.
Two rabbit and two rat studies with intramuscular injection of the vehicle or test item were reviewed by one pathologist. In rabbit studies (160 animals and 800 injection sites) intramuscular injections were given in the dorsolumbar, quadriceps femoris or gluteus medius muscles and each site received one injection. In rat studies (120 animals and 320 injection sites) injections were given in the quadriceps femoris or gluteus medius muscles and each site was injected 4 or 7 times. For both species, the injection was performed in the centre of this area, which was sampled at necropsy. Three pieces were trimmed from the sample, each piece separated by 0.3 cm in rats and 0.5 cm in rabbits. Piece 1 was in the middle, piece 2 lateral to it and piece 3 medial to it. Sections from each piece were re-examined and each of the original diagnoses, which summarized findings in all 3 sections together, was assigned to the section(s) where it was observed. The studies in each species were pooled but control and treated animals were evaluated separately.
Section 1 had a statistically significantly higher number of relevant findings when compared to sections 2 and 3 in treated rats and rabbits. In treated rats more than 85% of the original diagnostic features were found on this section. In rabbits, this percentage reached more than 75%. In control rats and rabbits, section 1 had a higher or equal number of findings than the other sections.
This study reveals that examination of more than one tissue piece does not greatly improve the quality of the diagnosis. One middle piece is considered to be sufficient to provide an accurate representation of lesions at intramuscular injection sites.

PS 649 ESTABLISHMENT OF T-CELL INDEPENDENT ANTIBODY RESPONSE IN RATS AS AN IMMUNOTOXICITY EVALUATION METHOD.

S. Ito, H. Hattori, R. Kawai, S. Komatsu, K. Muramatsu, M. Yagi, T. Matsuyama, T. Furukawa and A. Sanbuissho. *Medicinal Safety Research Laboratories, Daiichi Sankyo Co., LTD., Fukuroi, Shizuoka, Japan.* Sponsor: M. Teranishi.

T-cell independent antibody response (TIAR), as well as T-cell dependent antibody response (TDAR), plays an important role in the biological defense. In this study, we investigated the optimal conditions for evaluating TIAR in rats and performed a validation study of TIAR using well-known immunosuppressive agents, cyclophosphamide (CPA) and cyclosporine A (CsA).
To investigate the optimal conditions for TIAR, female Crl:CD (SD) rats were immunized with trinitrophenylated Ficoll (TNP-Ficoll) as a T-cell independent antigen. Based on the results, we selected the following conditions: the dose of TNP-Ficoll: 30 μg/rat; number of immunization: once; and timing of blood collection to measure anti-TNP antibodies by ELISA: 7 days after immunization.

To validate the TIAR model, female rats were administered orally CPA (2 or 6 mg/kg/day) or CsA (10 or 25 mg/kg/day) once daily for 14 days (Day 1 to 14), and immunized with TNP-Ficoll at 30 μg/rat on Day 8. Serum samples for measuring anti-TNP antibodies were collected on Day 15.
In CPA groups, decreases in anti-TNP IgM and anti-TNP IgG were observed. The lymphocyte subset analysis revealed decreases in mainly B cells in the peripheral blood and spleen, and decreased T cells in the thymus. CPA also decreased white blood cell parameters in hematology, and decreased spleen and thymus weights.
In CsA groups, decreases in anti-TNP IgM and anti-TNP IgG were also observed. The lymphocyte subset analysis revealed decreased T cells in the peripheral blood and thymus. In the hematology and organ weights, no significant changes were observed.
In conclusion, this TIAR model in rats will be useful for evaluating the effects on humoral immunity. Furthermore, a tiered approach using TDAR and TIAR may provide a more detailed immunotoxicity profile of drugs. In addition, the results of an additional study using other T-cell independent antigens will be reported.

PS 650 ANALYTICAL VALIDATION OF IMMUNOPHENOTYPING FOR CD5 IN PERIPHERAL BLOOD, SPLEEN, AND THYMUS FOR CD 1 MICE.

P. Sims[2], P. Joshi[2], J. E. Arrington[1] and J. Puchalski[2]. [1]*Toxicology Study Direction, Covance, Madison, WI and* [2]*Immunotoxicology, Covance, Madison, WI.*

The purpose of this study was to validate CD5 immunophenotyping in peripheral blood, spleen, and thymus for naïve CD 1 mice so it could be used as a substitute for CD3 when CD3 is the target molecule of immunomodulatory compounds. Accuracy, precision, stability, and antibody cocktail optimization endpoints were assessed for peripheral blood, spleen, and thymus for total T cells (CD5+/CD3+), helper T cells (CD5+4+/CD3+4+), cytotoxic T cells (CD5+8+/CD3+8+), B cells (CD5 19+/CD3-19+), natural killer (NK) cells (CD5 49b+/CD3-49b+), or thymus-specific T cell subsets, as appropriate. Two to three replicates from each sample were compared to evaluate inter and intra-assay precision and individual values from each animal were compared to assess variability. The assay was precise and accurate for blood, spleen, and thymus with most coefficient of variation (CV) values less than 10%. Results of stability testing indicated that all stained samples of fixed peripheral blood/single-cell suspensions were stable for up to 24 hours. The assay was linear for two antibody concentrations tested (1X and 2X) with most CV values less than 10%. Comparison of the immunophenotyping parameters for CD5 vs CD3 associated T, B, and NK cell populations in blood and spleen showed excellent correlation. Although the comparison in thymus was acceptable, the values were not as close as noted for peripheral blood and spleen due to slight differences in CD5 vs. CD3 ratios in the thymus for developing T cell populations. In summary, peripheral blood and tissue immunophenotyping methods using CD5 have been validated to accurately and precisely measure lymphocyte populations in peripheral blood, spleen and thymus. Furthermore, using CD5 as a substitute marker for CD3 yielded comparable lymphocyte populations in peripheral blood, spleen, and thymus, which validated its use for risk assessment.

PS 651 ANALYTICAL VALIDATION OF SPLEEN, THYMUS, AND MESENTERIC AND MANDIBULAR LYMPH NODE IMMUNOPHENOTYPING FOR SPRAGUE-DAWLEY RATS.

J. E. Arrington and P. Joshi. *Immunotoxicology, Covance, Madison, WI.*

The purpose of this study was to develop and validate tissue immunophenotyping procedures for Sprague Dawley rat spleen, thymus, and mesenteric and mandibular lymph nodes. These procedures can and have been used to evaluate changes in lymphoid tissue cell populations and were especially important when changes in lymphocyte populations were not detected in peripheral blood. The accuracy, precision, stability, and linearity of methods developed to measure total T cells (CD3+), T helper cells (CD3+4+), cytotoxic T cells (CD3+8+), B cells (CD3+45RA+), natural killer (NK) cells (CD3-161a+) in spleen and both lymph nodes and an array of T cell subtypes in the thymus (combinations of CD3±, CD4±, and CD8±) by flow cytometry were assessed. Three 1 cm3 sections of tissue were collected from each animal to evaluate all the study parameters. The assay was linear for all cell concentrations tested (10, 1, and 0.1 million input cells), except NK cells in the spleen and B cells in mandibular lymph nodes. Three separate tissue samples/organ were compared to determine accuracy and four replicate tests/sample were tested for precision. The assay was accurate and precise for spleen, thymus, and lymph nodes evaluation with most relative coefficient of variation values less than 10% (more variability with lymph nodes). Results of stability testing for whole tissue samples showed all parameters were stable up to at 24 hours, except cytotoxic T cells in mesenteric lymph nodes (due to slight variability). Stability for fixed single-cell suspensions was at least 24 hours for most parameters; CD3-4+8+ in the thymus and relative NK cells in the mesenteric and mandibular lymph nodes were not considered stable beyond the day of processing (linked to high initial values). In conclusion, tissue immunophenotyping methods have been validated and accurately and precisely measure lymphocyte populations in the spleen, thymus, and mandibular and mesenteric lymph nodes (although more variability is present with lymph nodes), but could be influenced by antibody concentration.

PS 652 *IN VITRO* MODEL FOR IMMUNOTOXICITY: SURFACE MARKER EXPRESSION AND CYTOKINE RELEASE IN NORMAL HUMAN DENDRITIC CELLS.

T. Landry, M. Klausner, A. Hunter, J. Sheasgreen, P. J. Hayden and S. Ayehunie. *MatTek Corp, Ashland, MA.*

Dendritic cells (DC) play a key role in the initiation of immune response by recognizing, internalizing, processing, and presenting antigens to T cells. However, DC function can be altered by environmental factors, pharmaceutical agents, and exogenous chemicals. These perturbations to immune cell function, i.e. immunotoxicity, can have significant effects on the natural defense mechanisms that ward off pathogens. While animal based assays are commonly used for immunotoxicity assessment, the Cosmetic Directive and REACH legislation bans the use of animals for many such studies. To examine the potential use of DC for immunotoxicity evaluation, large numbers of DC were generated from CD34+ progenitor cells and characterized for their expression of HLA-DR, CD1a, langerin, Birbeck granules, and co-stimulatory molecules (e.g. CD80, CD83, CD86, and CD40). In this study, we evaluated the effect of 3 immunotoxic compounds (ITC) on DC function. DC functionality was monitored by measuring surface marker expression and cytokine secretion following exposure to lipopolysaccharide (LPS). DC were first exposed to non-cytotoxic concentrations of the ITC for 24 hr and then to LPS for an additional 18 hr. FACS analysis of ITC-exposed DC showed effects in the rank order of immunotoxicity (severe to low effect): cyclosporine A > furosemide > verapamil as follows: a) decreased CD86 expression and b) dose-dependent decreases in the secretion of immuno-stimulatory cytokines including interleukin (IL)-12, IL-6, and IL-1β. Conclusion: DC expression of CD86 and release of immuno-stimulatory cytokines may serve as endpoints to predict immunotoxicity. Due to concerns with animal models in terms of cost, ethical issues, and relevance to hazard assessment in humans, the DC based assay could be an attractive in vitro model to predict immunotoxicity of compounds.

PS 653 USE OF AN ELECTROSPUN POLYCAPROLACTONE NANOFIBROUS SCAFFOLD AS A POTENTIAL DRUG DELIVERY SYSTEM FOR IMMUNOMODULATORY COMPOUNDS.

C. E. McLoughlin, M. J. Smith, W. Auttachoat, G. L. Bowlin and K. L. White. *Virginia Commonwealth University, Richmond, VA.*

Electrospun materials have potential use in many biomedical applications such as soft tissue replacements or as scaffolds to target drug delivery to local sites. Electrospinning is a polymer processing technique that can be used to create materials composed of fibers with diameters ranging from the micron to the nanoscale. Our laboratory has focused on investigating the effects of microfibrous and nanofibrous electrospun polycaprolactone (EPCL) on the immune system. These results have demonstrated that in both young (12 week) and old (6 month) mice, EPCL has minimal effect on various immune parameters including innate, humoral, and cell mediated immunity (CMI). With its lack of immunotoxicity, EPCL presents an excellent polymer scaffold for use in delivering drugs to local sites. Our recent studies focused on using EPCL nanofiber scaffolds with the known immunosuppressive compound dexamethasone (DEX) incorporated within the matrix. The ability of the EPCL-DEX scaffold to suppress CMI was evaluated using the delayed-type hypersensitivity (DTH) response to *Candida albicans* . Preliminary studies were conducted following subcutaneous implantation of a single disk (6-mm diameter) with 65, 100, or 200% w/w DEX in EPCL in the thigh region. Mice were sensitized with *C. albicans* following implantation and 8 days later were challenged with chitosan. Based on footpad swelling, dose responsive suppression of the DTH was observed at all dose levels. The animals that received the high dose (200%) had decreased spleen weights, however no change in spleen weight was observed at the lower doses (65 and 100%). These preliminary results suggest that implantation of a drug-containing electrospun scaffold may achieve local immunosuppression without systemic toxicity. Furthermore, EPCL may be an advantageous drug delivery system. Supported in part by NIEHS Contract ES 05454.

PS 654 TCDD ADSORBED ONTO NATURAL AND SYNTHETIC SILICA SUPPRESSES HUMORAL IMMUNITY IN MICE.

N. Kovalova[1, 2], R. Crawford[2], B. Kaplan[2], S. Kim[3], S. Boyd[3], B. Teppen[3], C. Johnston[5], T. Pinnavaia[4] and N. Kaminski[1, 2]. *[1]Pharmacology & Toxicology, Michigan State University, East Lansing, MI, [2]Center for Integrative Toxicology, Michigan State University, East Lansing, MI, [3]Crop & Soil Science, Michigan State University, East Lansing, MI, [4]Chemistry, Michigan State University, East Lansing, MI and [5]Soil Chemistry & Mineralogy, Purdue University, W. Lafayette, IN.*

2,3,7,8-tetracholordibenzo-p-dioxin (TCDD) is a well-characterized persistent environmental contaminant that exhibits suppression of primary humoral immune response in vivo and in vitro. Literature provides some evidence on mechanisms by which TCDD delivered in corn oil impairs IgM production by B cells. However, it is not well known if TCDD exposure under environmentally relevant conditions can lead to immunotoxicity. In present study, mice were treated with TCDD adsorbed on to synthetic amorphous silica with a mesocellular foam structure and on to natural silica. The surface area (385 m2g-1), pore volume (2.0 cm3g-1), and average window size (15 nm) of the synthetic silica allowed for the adsorption of TCDD as isolated single molecules on the pore walls. On day 3 of TCDD treatment mice were sensitized with sheep erythrocytes to initiate a humoral immune response. We found that TCDD adsorbed on to synthetic and natural silica is bioavailable as measured by the efficacy in suppression of anti-sRBC IgM antibody forming cell (AFC) response. Interestingly, TCDD produced similar level of suppression of anti-sRBC IgM AFC response regardless of the vehicle used to deliver TCDD. These results suggest that TCDD immobilized on various silicate phases in soils and sediments is bioavailable and able to cause immunotoxicity. (Supported in part by NIH P42 ES004911).

PS 655 TITANIUM DIOXIDE NANOPARTICLES: LACK OF IMMUNOSUPRESSIVE AND CONTACT HYPERSENSITIVITY EFFECTS IN FEMALE MICE.

W. Auttachoat[1], C. E. McLoughlin[2], M. J. Smith[1] and K. L. White[1]. *[1]Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA and [2]Biomedical Engineering, Virginia Commonwealth University, Richmond, VA.*

Titanium dioxide is a white pigment widely used in products such as paints, food, and cosmetics. US FDA regulates its use as a food color additive at levels not to exceed 1% by weight. Titanium dioxide nanoparticles (NTI) have been reported to induce liver injury and to decrease interleukin-2 production following oral exposure for 30 days in mice. However, it remains unclear whether exposure to NTI (< 25 nm diameter) results in immunosuppressive effects (in B6C3F1 mice) or induces a contact hypersensitivity response (in BALB/c mice). In these studies, immunosuppression was evaluated using the Plaque Assay (TDAR) to sheep erythrocytes (sRBC) and the Delayed-Type Hypersensitivity Assay (DTH). Contact hypersensitivity was evaluated using the Local Lymph Node Assay (LLNA), Irritancy Response Assay (IRR), and the Mouse Ear Swelling Test (MEST). After 28 days of oral gavage, NTI (1.25-250 mg/kg in 0.5% methylcellulose) produced no significant effects on the TDAR or on the DTH response to C. albicans. Furthermore, there were no effects on the weights of the major immune organs (spleen, thymus). Liver, kidney, and lung weights were also unaffected. Following dermal exposure for 3 days, NTI (2.5% to 10% w/v in 4:1 acetone olive oil) did not affect auricular lymph node cell proliferation (LLNA), nor was an irritancy response produced (IRR), when compared to the vehicle control. Dermal sensitization (2.5-10%) on the back and subsequent challenge (10%) on the right ear with NTI produced no significant effects on percent ear swelling when compared the vehicle irritancy control group in the MEST. In summary, these results demonstrate that oral exposure to NTI at doses up to 250 mg/kg was not immunosuppressive in B6C3F1 mice in two functional holistic assays (TDAR, DTH). Furthermore, dermal exposure to NTI (up to 10% w/v) did not induce contact hypersensitivity in BALB/c mice. Supported in part by NIEHS Contract ES 05454.

PS 656 ACTIVATED CYTOTOXIC AND HELPER T-CELLS ARE REDUCED IN C57BL6 MICE EXPOSED TO TUNGSTATE IN A ONE-GENERATION EXPOSURE.

A. R. Osterburg[1], C. T. Robinson[2], V. P. Mokashi[1], M. G. Stockelman[1] and G. F. Babcock[2]. *[1]Naval Medical Research Unit - Dayton, Wright-Patterson AFB, OH and [2]Shriners Hospitals for Children and the University of Cincinnati, Cincinnati, OH.* Sponsor: K. Mumy.

Tungstate has been identified as a ground water contaminant at military firing ranges and can be absorbed by ingestion. In this study, C57BL6 mice were exposed to sodium tungstate (Na2WO4•2H2O) in their drinking water in a one-generation model. Previous work in the laboratory demonstrated a reduction in activated cytotoxic and helper T-cells after 28 and 90-day exposures. Drinking water included weight adjusted tungstate concentrations of 0, 2, 62.5, 125, and 200 mg/kg/day. Twenty-four hours prior to euthanasia, mice were given (i.p.) saline, LPS (5 mg/kg), or SEB (20 μg) for controls and to stimulate innate and adaptive immune responses, respectively. After euthanasia splenocytes, blood, bone marrow and thymus were collected and stained with lymphocyte and/or myeloid immunophenotyping panels and analyzed by flow cytometry. In the one-generational exposure, we observed statistically significant reductions in the quantities of cytotoxic T-cells (CD3+CD8+CD71+) and helper T-cells (CD3+CD4+CD71+) in SEB treated mice. Cytotoxic T-cells were 7.98% +/- 0.49 SE and 6.33% +/- 0.49 SE for P and F1 mice in 0 mg/kg/d tungstate vs. 1.58% +/- 0.23 SE and 2.52% +/- 0.25 SE in the 200 mg/kg/d of tungstate (P<0.001). Similarly, helper T-cells were reduced 6.21+/- 0.39 SE and 7.20% +/- 0.76 SE for the 0 mg/kg/d, and 2.28 +/- 0.41 SE and 2.85+/- 0.53 SE for the 200 mg/kg/d tungstate groups (P<0.001). Tungstate-dependent differences where only observed in spleens of exposed animals. There were no statistically significant differences in immune responses due to tungstate exposure and generation. These data suggest that tungstate exposure may alter the responses of cytotoxic and helper T-cells that may in turn alter the susceptibility of exposed individuals to infection.

PS 657 ASSOCIATIONS OF PERFLUOROCHEMICALS (PFCS) WITH IMMUNE, BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN BOTTLENOSE DOLPHINS.

P. Fair[1], T. Romano[2], T. Hulsey[3], J. Reif[4], G. Bossart[5], J. Adams[1], M. Houde[6], D. Muir[6], C. Rice[7] and P. Margie[8]. *[1]NOAA/NOS, Charleston, SC, [2]Mystic Aquarium, Mystic, CT, [3]Medical University of South Carolina, Charleston, SC, [4]Colorado State University, Fort Collins, Co., [5]Harbor Branch Oceanographic Institute at Florida Atlantic University, Fort Pierce, FL, [6]Environment Canada, Burlington, ON, Canada, [7]Clemson University, Clemson, SC and [8]University of Nevada Las Vegas, Las Vegas, NV.*

Despite the widespread occurrence of Perfluorochemicals (PFCs), very little is known regarding the impact of these contaminants on the health of wildlife populations. This study, therefore, investigated the relationship between PFCs and health/immune parameters in two populations of Atlantic bottlenose dolphins. A repeated cross-sectional comprehensive health assessment study was conducted during the summers of 2003-2005 near Charleston, SC (CHS; n=80) and the Indian River Lagoon, FL (IRL; n=82). Correlative data analysis stratified by site examined relationships between PFC levels in serum and immune, biochemical and hematology parameters. Significant relationships between immunological measures were observed with ∑PFC, as well as individual PFCs including PFOS. B cell proliferation and numbers of CD2+, CD4+, CD19+, CD21 and MHCII+ cells were positively correlated with PFC levels in CHS dolphins. Similar results were observed for IRL dolphins with two of these parameters: B cell proliferation and numbers of CD21+ cells. PFC levels were also positively associated with lymphocyte numbers, CPK, ALP, cholesterol, TIBC, iron, and triglycerides and negatively with eosinophils in CHS dolphins. ALT, ALP, BUN, BUN/creatine, Ca, CPK, and LDH were positively correlated with PFC levels in IRL dolphins. Serum proteins exhibited negative relationships with PFC concentrations. Alterations in both the number of parameters and significance level associated with PFCs were higher in CHS dolphins likely corresponding with their higher PFC concentrations. Bioaccumulation of PFC contaminants in dolphins may modulate immune responses and alter hematological and serum analytes and pose a threat to the health and viability of these populations.

PS 658 COMPARISON EFFECTS OF *IN VITRO* PFOS EXPOSURE ON BOTTLENOSE DOLPHIN AND MURINE IMMUNE FUNCTION.

J. R. Wirth[1], M. M. Peden-Adams[2], N. Henry[3] and P. A. Fair[3]. *[1]Graduate Program of Marine Biology, College of Charleston, Charleston, SC, [2]Harry Reid Center for Environmental Science, University of Nevada - Las Vegas, Las Vegas, NV and [3]NOAA/NOS/CCEBHR, Charleston, SC.*

The effects of emerging contaminants on the immune system of mammals are poorly understood, with limited information available about the immunotoxicological effects of PFOS at environmentally relevant exposures. Marine mammals are protected species; thereby, prohibiting the direct assessment of the effects of contaminant exposure. Previous studies in our lab have shown that PFOS modulates

immune function in mice and correlates with many immune parameters in bottlenose dolphins. Due to challenges of working with wild dolphins, this study assessed the effects of *in vitro* exposures to PFOS on immune functions in dolphins and mice to determine the predictability of *in vitro* effects to *in vivo* observations as part of a parallelogram approach to assessing risk. Bottlenose dolphin peripheral blood leukocytes and B6C3F1 mouse splenocytes were exposed to PFOS in culture at 0, 10, 50, 100, 500, 1000 or 5000 ng/mL and NK cell activity and lymphocyte proliferation were assessed. NK cell activity was modulated, decreasing at dosages of 10, 50, 100, 500 and 1000 ng/mL and increasing at the 5000 ng/mL dose as compared to control in both species. Mitogen-induced lymphocyte proliferation was significantly decreased in murine cells, but not in dolphin cells. Using both dolphin and murine cells provides a comparative approach for evaluating the effects of PFOS on immune functions.

PS 659 THE ROLE OF T-CELL IL-2 AND MACROPHAGE IL-10 PRODUCTION IN PFOS-INDUCED HUMORAL IMMUNOSUPPRESSION.

M. M. Peden-Adams[1], J. Berger-Ritchie[2, 1], S. Young[3], N. Ayala[4] and D. Keil[5]. *[1]Harry Reid Center for Enivronmental Studies, University of Nevada-Las Vegas, Las Vegas, NV, [2]School of Community Health, University of Nevada-Las Vegas, Las Vegas, NV, [3]Department of Anthropology, University of Nevada-Las Vegas, Las Vegas, NV, [4]Clinical Laboratory Sciences, University of Nevada-Las Vegas, Las Vegas, NV and [5]Department of Medical Laboratory Sciences, School of Medicine, University of Nevada-Las Vegas, Salt Lake City, UT.*

Perfluorinated hydrocarbons have been manufactured for over 40 years and have numerous applications in industry. This group of compounds has recently generated much interest as some of these compounds (i.e., perfluorooctane sulfonate (PFOS)) are persistent in the environment and are detectable in blood samples of both wildlife and humans. Studies show that these perfluorinated compounds cause various toxicological effects; however, effects on immune function have not been addressed at length. We have previously shown decreased SRBC-specific IgM production in adult B6C3F1 mice following exposure to PFOS. The current study assessed possible modes of action for this suppression. Adult female B6C3F1 mice were exposed orally to PFOS for 28 days. Numbers of CD4+/CD25+/CD70+, CD4+/CD25+/CD27+, CD4+/CD27+/CD70+, CD19+/CD25+/CD70+, CD19+/CD25+/CD27+, CD19+/CD27+/CD70+, and C19+/IgM+ cells were not altered with or without SRBC injection. Peritoneal macrophage secretion of IL-1 was not altered with or without SRBC injection. Macrophage IL-10 production was suppressed by PFOS following SBRC injection but not in resting cells not challenged with SRBC. CD4+ T-cell production of IL-2 was suppressed in resting cells following PFOS exposure, but suppression was not observed following SRBC challenge. These data indicate that expression of the selected cell surface activation markers and T-cell IL-2 production my not be associated with PFOS-induced SRBC-specific IgM production in this rodent model, but that characterization of events related to IL-10 production by macrophages is warranted.

PS 660 THE T CELL DEPENDENT ANTIBODY RESPONSE AS A PREDICTOR OF THE TYPE OF IMMUNOSUPPRESSION ASSOCIATED WITH PERIPHERAL MULTIFOCAL LEUKOENCEPHALOPATHY.

E. R. Fedyk, P. F. Smith and V. J. Kadambi. *Drug Safety Evaluation, Millennium Pharmaceuticals Inc., Cambridge, MA.*

Peripheral multifocal leukoencephalopathy (PML) is a rare, opportunistic viral disease that is characterized by progressive destruction of the white matter of the brain at multiple locations, which is caused by lytic infection of myelin-producing oligodendrocytes. A majority of healthy adults are latently infected with the causative agent, the JC virus. PML occurs almost exclusively in patients with immune deficiencies, such as acquired immune deficiency syndrome, X-linked agammaglobulinemia, common variable immunodeficiency, and acute lymphoblastic leukemia which prompted investigators to conclude that PML is caused by impaired adaptive immunity to the JC virus. The recent voluntary withdrawls of approved therapeutics prompted us to conduct a meta-analysis of the mechanism of action of small molecules and biologics that are associated with PML. This analysis revealed that a activity shared by each of these therapeutics is impairment of the adaptive immune system. The International Conference on Harmonisation (ICH) Guidance S8, "Immunotoxicity Studies for Human Pharmaceuticals," provides guidance on assessing the potential effects of therapeutics on the adaptive immune system. It specifically endorses the T cell-dependent antibody response (TDAR) as a cumulative assessment of adaptive immune function. Our subsequent meta-analysis investigated if agents which target the adaptive immune system and are associated with PML, inhibit nonclinical and/or clinical TDAR. These results illustrate that nonclinical impairment of TDAR correlates with impairment of clinical TDAR and PML. The authors thus conclude that impairment of TDAR responses is predictive of the type of immune suppression that is associated with PML. It should be noted nonetheless that while suppression of adaptive immunity is required for the development of PML, it alone is insufficient. Additional predictors of PML are needed for more accurate risk assessment.

PS 661 IMMUNOTOXICOLOGIC EVALUATION OF RITUXIMAB AND CYCLOSPORINE IN CYNOMOLGUS MONKEYS.

J. Legrand[1], E. Grosdidier[1], R. Fleurance[1], C. Mimouni[1], A. N'Guyen[1], F. Gervais[1] and J. Descotes[2]. *[1]CIT, Evreux, France and [2]Poison Center, Lyon, France.*

The immunotoxicologic effects of the monoclonal antibody rituximab (RTX) and the immunosuppressive agent cyclosporine (CSA) have been tested in Cynomolgus monkeys. Male and female animals were treated for 4 weeks either once weekly by slow intravenous infusion with saline or 10 mg/kg RTX, or daily by oral gavage with olive oil or 100 mg/kg CSA. KLH (1 mg) was injected IM on days 14 and 21, and blood samples were taken on days 1, 21 and 28 for the determination of anti-KLH IgM and IgG levels by ELISA. Lymphocyte subsets from blood samples drawn before treatment and on day 28 were analyzed by flow cytometry, and those from selected lymphoid organs taken on day 29 by cytometry and immunohistochemistry. No mortality, clinical signs, or overt changes in the weight of lymphoid organs were observed. Histologic examination showed clear effects of both compounds on the spleen consisting of a marked decrease in germinal center development. There was also a marked decrease in the marginal zone of the spleen in RTX-treated animals confirmed by anti-CD19 immunolabeling as compared to controls, and only a minimal increase in CSA-treated animals. No consistent changes were seen in the thymus and lymph nodes of RTX-treated animals. In contrast, thymus atrophy and marked decreases in germinal center development and paracortical areas in the lymph nodes confirmed by anti-CD3 and anti-CD19 immunolabeling were observed in CSA-treated monkeys. Anti-KLH IgM and IgG levels were either not different from control animals or decreased on all occasions in some RTX-treated animals, whereas IgM and IgG responses were consistently decreased in CSA-treated monkeys. Lymphocyte subset analysis found decreased B cell numbers in the blood of all animals treated with either compound. Less B cells were seen in the lymph nodes of RTX- or CSA-treated animals than in controls with a more pronounced effect after RTX treatment.

PS 662 DISCHARGING A THEORETICAL RISK OF INTEGRASE INHIBITOR-INDUCED IMMUNOTOXICITY IN JUVENILE RATS.

M. Rhodes[1], S. Laffan[2], C. Genell[2], J. Gower[2], C. Maier[2], G. Nichols[1], T. Fukushima[3], J. Chism[1], G. Tabolt[1] and A. Bassiri[2]. *[1]GlaxoSmithKline, Research Triangle Park, NC, [2]GlaxoSmithKline, King of Prussia, PA and [3]Shionogi Pharmaceuticals, Osaka, Japan.*

HIV-1 integrase inhibitors (INI) are a promising class of antiretrovirals for the treatment of HIV, and because of the anticipated clinical efficacy, there is particular interest in expanding their use into pediatric populations. A theoretical concern for developmental immunotoxicity was raised after a publication suggested that 2 HIV INI compounds (p8 [5CITEP] and p10 [L-708,906]) have activity on recombination activating genes 1 and 2 (RAG1/2) in vitro. These genes are required for assembly of immunoglobulin and T cell receptors (TCR), and therefore development of mature B and T lymphocyte populations. The potential effects of the integrase inhibitor GSK1349572 on RAG1/2 were addressed by developing assays in juvenile rats to measure TCR Vbeta 8.2/8.4, 8.5, 10, and 16 usage by flow cytometry (as an indicator of TCR repertoire diversity), and a T cell dependent antibody response (TDAR) (as an indicator of immunosuppression). These endpoints were incorporated into a rat juvenile toxicity study along with immunophenotyping, hematology, and histopathology. Doses of 0, 0.5, 2, or 75 mg/kg/day were given from Day 4 to 66 postpartum. There were no test article-related effects on immunologic competence as measured by TDAR (Days 50 and 59 postpartum), and no effects on lymphocyte subsets (T cells, both CD4 and CD8 subsets, and B cells), or CD4 and CD8 T cell receptor Vbeta usage in peripheral blood. Histopathology of immunological organs (spleen, thymus, lymph nodes) and hematology evalua-

tion revealed no test article-related effects. The no observable adverse effect level (NOAEL) for immunotoxicity endpoints was 75 mg/kg/day. The data demonstrate that there were no GSK1349572-mediated alterations in T and B cell number or the diversity of the T cell repertoire and no effect on immune responsiveness in a TDAR, and therefore suggest no unusual compound-specific risk of developmental immunotoxicity in pediatric clinical studies.

PS 663 THE EFFECTS OF TRIBUTYLTIN (TBT) ON SPLENOCYTES IN F1 RATS EXPOSED TO TBT *VIA* PLACENTA, THEIR DAM'S MILK, AND/OR FOOD.

M. Tsunoda[1], T. Kido[1], R. Ikeuchi[1], T. Kaido[1], M. Hosokawa[2, 1], C. Sugaya[1], H. Ohta[1], H. Katagiri[3], K. Hayashi[4], T. Kosaka[4], Y. Takeuchi[5], T. Tashiro[5] and Y. Aizawa[1]. *[1]Preventive Medicine and Public Health, Kitasato University School of Medicine, Sagamihara, Japan, [2]Juntendo University Faculty of Medicine, Tokyo, Japan, [3]Kitasato University School of Allied Health Sciences, Sagamihara, Japan, [4]Institute of Environmental Toxicology, Tokyo, Japan and [5]Aoyama Gakuin University School of Science and Engineering, Sagamihara, Japan.*

Immunotoxicity is one of the major toxic effects of tributyltin (TBT) as well as neurotoxicity. Immunotoxicity is stronger on F1 rats exposed to TBT via placenta and dam's milk than adults. In our previous study, neurotoxic effects of TBT on F1 rats was observed and remained after cessation of exposure. Whether or not the immunotoxicity of TBT remains after cessation remains to be clarified. In this study, TBT immunotoxicity in F1 rats at 9 wk was evaluated by determining TNFα in the supernatant of the splenocytes. Pregnant Wistar rats were exposed to TBT at 0 and 125 ppm in their food. The F1 rats were exposed to TBT via the placenta and their dam's milk. After weaning, exposure to TBT in the F1 rats was stopped. At 6 wks of age, the rats were randomly assigned to either the control or the group fed a diet containing 125 ppm of TBT. The F1 rats were divided into the control-control (CC) group; the TBT-control (TC) group, exposed to TBT via placenta and dam's milk; the control-TBT group, exposed to TBT via food; and the TBT-TBT (TT) group, exposed to TBT via placenta, dam's milk, and food. At 9 wks, spleens were excised and cell suspensions of splenocytes were prepared. Concentrations of TNFα in the supernatant of activated splenic macrophages, T cells and B cells, were determined by ELISA. Mean body weights in the TC and TT groups were significantly lower than that in the CC group. Mean values of TNFα in the supernatant of B cells were higher in the TC and TT groups. There were no significant differences for TNFα in the supernatant of macrophages or the T cells. The effects of TBT on the splenocytes in the F1 rats exposed to TBT via the placenta and their dams' milk may continue after cessation.

PS 664 USE OF TDAR (SRBC) IMMUNOTOXICITY MONITORING IN AN EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY TEST WITH LEAD ACETATE.

J. Hargitai[1], D. J. Esdaile[1], R. Kubaszky[1], T. Hanley[2], D. Minnema[2] and R. Lewis[3]. *[1]Lab. Research Ltd., Veszprem, Hungary, [2]Syngenta Crop Protection LLC, Greensboro, NC and [3]Syngenta, Jealotts Hill, Bracknell, United Kingdom.*

The "Extended one-generation reproduction study" in rats as proposed by draft OECD guideline and the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI), requires evaluation of immunotoxicity in a functional antibody response assay. In the main study, Lead Acetate was provided in drinking water at 0, 100, 800 and 1700 ppm to F0 adult Wistar rats throughout pre-mating, mating, gestation, and lactation, and to the F1 generation to adulthood. A TDAR, sheep RBC (sRBC) assay was performed in the F1 generation subset designated for immunotoxicity assessment following a single iv injection of standardised sRBC (2x10^8/rat) between PND63-67. These animals were sacrificed 6 days later and serum prepared. Samples were analysed in a commercial ELISA kit for rat anti-sRBC IgM levels (Life Diagnostics Inc., PA, USA). The procedure was shown to be sensitive for the detection of immunosuppressive effects in a validation study and in a concurrent positive control group in the main study. Age-matched rats of each sex were treated with cyclophosphamide at 30 mg/kg bw/day for 5 consecutive days, then serum prepared on the 6th day. The positive control response was strongly and consistently reduced. There was no clear statistically significant difference between negative control and treated groups, although there was an indication of a trend to a lower response in male rats treated with Lead Acetate. This outcome showed that although Lead is considered to exhibit immunosuppression in some experimental models, the TDAR sRBC assay in the adult F1 rats in this protocol did not show a strong immunosuppressive response. However, it was shown that it is practicable to include a valid TDAR assay into this integrated developmental neurotoxicity, immunotoxicity and reprotoxicity protocol.

PS 665 LONG-TERM IMMUNOTOXIC EFFECTS OF COMBINED PRENATAL AND NEONATAL ATRAZINE EXPOSURE IN BALB/C MICE.

I. Holaskova, R. Schafer, K. Brundage, E. Lukomska and J. B. Barnett. *Microbiology, Immunology, and Cell Biololgy, West Virginia University, Morgantown, WV.*

Atrazine & its metabolites are present at high levels in a high percentage of water supplies in agro-intensive areas. Given that humans in these areas use water from these contaminated sources, we investigated the long-term effects of prenatal & neonatal exposure to atrazine on the immune system. To determine these effects, pregnant Balb/C dams were exposed to 700 μg atrazine per day from day 10 post-coitus to day 10 postpartum. All offspring were allowed to nurse their natural mother & were weaned at 25 days of age. Offspring were randomly segregated by sex & exposure & aged to approximately 1 year of age at which time their spleens were removed for analysis. Phenotypic analysis was performed using flow cytometry & cytokine production was measured by cytometric bead array after anti-CD3/anti-CD28 stimulation of the spleen cells.. The spleen cells were stained to determine total T cells, CD8+ T cells, CD4+ T cells, B cells, granulocytes, macrophages, NK cells, nTreg & LAG-3+-Treg cells. Cytokines measured include interleukin (IL)-2, IL-4, IL-6, interferon-γ, tumor necrosis factor-α, IL-17A, IL-10 & TGF-β1 which measures the functional ability of the cells to respond to stimulation as well as allows for further classification of the T cells into TH1, TH2, TH17 cells. One year old female offspring had significant decreases in the percentage of CD8+ T cells & significant increases in granulocytes & NK cells. There was a trend (same type of change; 1 of 2 runs significant) towards an increase in LAG-3+ Treg cells. Male offspring showed significant decreases in the percentage of CD8+ T cells & significant increases in CD4+ as well as a trend towards a significant increase in NK cells. This is the first time the long term changes in immune cell phenotype have been documented after a prenatal & neonatal exposure to atrazine & it demonstrates that these early life exposures can result in permanent changes to the immune system. Supported by NIH grants ES014698, RR016440 & RR020866

PS 666 EFFECTS OF CIGARETTE SMOKE ON INFECTIOUS BURDEN, RESPIRATORY INFECTION AND INFLAMMATORY RESPONSE WITH CHLAMYDIA PNEUMONIAE IN A MOUSE MODEL.

S. P. Doherty-Lyons[1], C. Hoffman[1], S. Kumar[2], S. Kolhoff[2], M. R. Hammerschlag[2] and J. T. Zelikoff[1]. *[1]Environmental Medicine, New York University School of Medicine, Tuxedo, NY and [2]Pediatrics, State University of New York Downstate, Brooklyn, NY.*

Chlamydia pneumoniae (Cpn) is a ubiquitous pathogen that causes acute respiratory disease, particularly in children. No data currently exist on the effect of smoking on the course of Cpn respiratory infection. Given the alterations in the immune status of children following exposure to mainstream cigarette smoke (MCS) and the increased incidence of lower respiratory tract infection (LRTI) following CS exposure in young children, we hypothesized that exposure of juvenile C57BL/6J mice to MCS would increase the severity and duration of Cpn infection and alter airway responsiveness. Mice were exposed to MCS for either 1 or 2 weeks, followed by intratracheal instillation with Cpn (1x10^5 IFU/ml). Animals were then sacrificed at days 13-14 and 25-28 post-infection (PI). Cpn burdens in the lungs were determined using rtPCR and found to be similar in both air and MCS-exposed mice in both sets of experiments. Chronic pulmonary inflammation was noted in all Cpn-infected mice at both PI time points, but was most prominent at 25-28 days PI in both treatment groups. In those mice exposed for 2 weeks to MCS, significant differences between the groups were seen in serum cytokine levels (IL-4 and IFN-g),but only at the later timepoint (day 25 PI). There was also a shift in the ratio of IFN-g/IL-4 over time towards a Th2 bias in both experiments, which could be associated with the increased pulmonary inflammation seen here at day 25 PI. The worsening Cpn-induced inflammation that followed infection in the absence of increased bacterial burdens, and the shift to a Th2 bias in the MCS-exposed animals suggests that cigarette smoke (at least in part) may contribute to the exacerbation of injury; alterations of the T-cell response important in the resolution of some adverse lung lesions may be responsible for such effects. Supported by a Sub-contract with SUNY Downstate Med Ctr.

PS 667 EVALUATION OF THE IMMUNOMODULATORY POTENTIAL OF DIETHYL PHTHALATE FOLLOWING DERMAL EXPOSURE IN A MURINE MODEL.

J. L. Franko, E. Lukomska, B. J. Meade and S. E. Anderson. *NIOSH, Morgantown, WV.*

Over the past 50 years the prevalence of asthma and allergy has increased throughout the US and other industrialized nations. During the same period there has been an increase in the production and use of phthalate compounds. Previous studies have suggested that certain phthalate compounds, including diethy-hexyl phthalate and butyl benzyl phthalate, have contributed to allergic disease by functioning as adjuvants, augmenting immunologic responses to other chemical and protein antigens without being allergenic themselves. Diethyl phthalate (DEP), another phthalate ester, used as a plasticizer in a variety of consumer products, has the potential for widespread exposure, both occupationally and in the general population. Although DEP is not an immune sensitizer, little is known about other immunological effects that may be associated with DEP exposure. The current study evaluated the immunotoxic potential of DEP. Dermal exposure to DEP concentrations as low as 50% were shown to be immunosuppressive, resulting in a modest reduction in thymus weights (%) and numbers of CD4+ and CD8+ thymocytes (30.7 and 37.6% respectively) in BALB/c mice. A dose responsive decrease in the primary IgM response to sheep red blood cells was observed following dermal exposure to DEP reaching 54% reduction at the highest concentration. Conversely, prior dermal exposure to 75% DEP enhanced airway hyperreactivity responses to aerosolized methacholine in mice sensitized to increasing concentrations of OVA, suggesting that despite its immunosuppressive properties, DEP may enhance airway responsiveness to environmental and occupationally relevant antigens and thereby contributing to the increased prevalence of allergic disease. These results warrant the need for additional investigation into the mechanisms underlying the immunotoxic effects associated with DEP exposure.

PS 668 AFRICAN DUST ENDOTOXINS AND THEIR PRO-INFLAMMATORY ROLE IN HUMAN BRONCHIAL EPITHELIAL CELLS.

M. G. Ortiz-Martínez[1, 3], E. Rivera[4, 3], L. B. Méndez[2] and B. D. Jiménez Vélez[1, 3]. [1]*Biochemistry, University of Puerto Rico- Medical Sciences Campus, San Juan,* [2]*Department of Microbiology and Molecular Genetics, University of California, Irvine, CA,* [3]*Center for Environmental and Toxicological Research, University of Puerto Rico, San Juan and* [4]*Department of Biology, University of Puerto Rico-Río Piedras, San Juan.*

African dust reaching the island of Puerto Rico is associated with increments in ambient particulate matter (PM). Among the PM components associated with adverse health effects are particle-bound endotoxins (ENX). Therefore, aqueous and organic extracts of PM_{10} samples collected from specific African Dust Events (ADE) reaching Puerto Rico in March 2004 were characterized for their ENX content and evaluated for its cytotoxic and pro-inflammatory responses in the human bronchial epithelial cell line BEAS-2B. To accomplish these aims, we analyzed PM_{10} extracts from samples collected at the Atlantic Ocean and two sites in Puerto Rico (e.g. rural vs. urban). The ENX content in the extracts, as determined by the LAL assay, was higher in the organic fraction of PM_{10} and increased during ADE for all sites. Organic extracts of the ADE samples were highly cytotoxic even at low doses. The levels of IL-6 and IL-8 in cell culture supernatants were significantly higher in cells exposed for 24 hrs to ADE PM_{10} aqueous extracts versus those exposed with non-ADE extracts. However, only IL-8 was induced with the ADE organic extracts. To evaluate the role of ENX in the induced cellular responses, the extracts were pretreated with the ENX inhibitor polymyxin B prior cell exposure and the induction of pro-inflammatory mediators in human lung cells was inhibited. Since inhibition of ENX reduced the pro-inflammatory responses of ADE PM_{10} extracts current experiments are being performed to determine the expression of Toll-like receptor 4, which recognizes ENX, after exposure to PM extracts. The results obtained so far suggest that African dust reaching Puerto Rico increases the ENX content in ambient PM, which can induce inflammatory responses in the lung.
Supported by MBRS-RISE Grant R25GM061838.

PS 669 AFRICAN DUST $PM_{2.5}$ REACHING PUERTO RICO INDUCES OXIDATIVE STRESS AND PRO-INFLAMMATORY MEDIATORS IN HUMAN LUNG CELLS.

R. I. Rodríguez[1, 3], L. B. Méndez Torres[2] and B. D. Jiménez Vélez[1, 3]. [1]*Biochemistry, University of Puerto Rico-Medical Sciences Campus, San Juan, Puerto Rico,* [2]*Department of Microbiology and Molecular Genetics, University of California, Irvine, CA and* [3]*Center for Environmental and Toxicological Research, University of Puerto Rico, San Juan.*

African dust reaching the island of Puerto Rico is known to sporadically increase ambient particulate matter (PM). Particle-bound metals have been associated with adverse health effects. Organic extracts from $PM_{2.5}$ samples collected during African dust events (ADE) reaching Puerto Rico were characterized for its metal content and oxidant capacity. Extracts were also evaluated for its role in inducing oxidative stress and pro-inflammatory responses in the human bronchial epithelial cells (BEAS-2B). ADE and non-ADE $PM_{2.5}$ organic extracts exhibited similar oxidative capacity, using the DTT assay. However, metal compositions of organic extracts, determined by ICP-MS analysis revealed an increase in Cu and V concentrations in ADE vs. non-ADE extracts. This supports the hypothesis that differences in metal concentrations might be responsible for the immune responses observed between extracts. In-vitro experiments showed a reduction in the total antioxidant capacity and glutathione concentrations in lysates of organic extracts (50µg/ml). This reduction was greater in ADE organic extracts. Secretions of IL-6 and IL-8 were significantly higher in cells exposed (24hrs) to ADE vs. non-ADE extracts. However, the levels of MCP-1 were significantly reduced after exposure to either type of extract. The addition of metal chelators (deferoxamine and tetraethylenepentamine) increased antioxidant capacity and decreased the secretion of pro-inflammatory mediators in human lung cells exposed to organic extracts. These results demonstrate that the effects of organic extracts are produced by trace elements. This study suggests that African dust reaching Puerto Rico increases the concentrations of trace elements in ambient $PM_{2.5}$, inducing oxidative stress and inflammatory responses in the lung. Supported by MBRS-RISE Grant R25GM061838

PS 670 HEALTH EFFECTS DUE TO ACUTE EXPOSURE OF DUST SAMPLES COLLECTED FROM NELLIS DUNES RECREATIONAL AREA.

S. Proper[1], M. Peden-Adams[2], N. Ayala[2], J. Berger-Ritchie[2], S. Labahn[2], S. Young[2], B. Buck[2], D. Goossens[2, 3], D. Soukup[4], R. Sudowe[2], Y. Teng[2], D. Baron[2], J. R. Harkema[1] and D. E. Keil[5, 2]. [1]*Michigan State University, East Lansing, MI,* [2]*University of Nevada, Las Vegas, NV,* [3]*Katholieke Universiteit, Leuven, Belgium,* [4]*California State University, Bakersfield, CA and* [5]*University of Utah, Salt Lake City, UT.*

The Nellis Dunes Recreational Area (NDRA; Las Vegas, NV) is a popular natural site for recreational vehicle operators with over 300,000 visitors per year. Potential health effects caused by PM10 generation from ORV activity were examined. B6C3F1 mice were exposed to dust collected from 3 NDRA map unit sites (2.2, 3.1, or 3.2). Exposure was administered via oropharyngeal aspiration at doses of 0.1, 1, 100, and 1000 mg/kg/day for 3 days. The lowest adverse effect level (LOAEL) for map unit 2.2 was 0.1 mg/kg/day while the LOAEL was 1.0 mg/kg/day for map units 3.1 and 3.2. Each LOAEL was based on significant and dose-responsive decreases in specific IgM B-cell responses and CD4/CD8 splenic lymphocytic subpopulations. Pulmonary histopathology was evident in the 100 and 1000 mg/kg/day dose groups. Observations were primarily multifocal, centriacinar bronchiolitis with accumulation of dust-filled macrophages and interstitial fibrosis. Alveolar bronchiolarization and bronchiolitis obliterans were also common, characteristic of pneumoconiosis. Median particle diameter for map units 2.2, 3.1, and 3.2 was 4.3, 2.4, and 3.1 um, respectively. The predominant mineral was smectite while palygorskite was also common. ICP-MS measured >20 naturally-occurring metals to include: 23-143 ppm total As, 18-24 ppm Pb, 9-18 ppm Cr, 6600-9800 ppm Al, 106-183 ppm Sr, and 274-428 ppm Mn. Research is needed to determine the impact of dust exposure on respiratory health and disease resistance because of the likelihood of human exposure.

PS 671 INFLAMMATION AND TISSUE DAMAGE IN THE MOUSE LUNG INDUCED BY WOOD SMOKE PARTICULATE MATTER FROM NEW AND OLD TECHNOLOGY HEATING APPLIANCES.

O. Uski[1], M. Happo[2], P. Jalava[1, 2], K. Joachim[3], T. Brunner[3, 4, 5], I. Obernberger[3, 4, 5], M. Tapanainen[1], J. Jokiniemi[2, 6] and M. Hirvonen[1, 2]. *[1]National Institute for Health and Welfare, Kuopio, Finland, [2]University of Eastern Finland, Kuopio, Finland, [3]BIOENERGY 2020+ GmbH, Graz, Austria, [4]Graz University of Technology, Graz, Austria, [5]BIOS BIOENERGIESYSTEME GmbH, Graz, Austria and [6]VTT Technical Research Centre of Finland, Espoo, Finland.* Sponsor: M. Viluksela.

Particulate air pollution (PM) is a global problem, which is responsible for large variety of adverse health outcomes. EU has decided to increase the use of biomass energy to 20% on average from the total energy consumption by 2020. Since large proportion of PM emissions are derived from small scale combustion, it is important to reveal the effects of the combustion technology, user practice and fuel type or quality on these emissions. The effects of these factors on toxicological properties are still poorly known. In this study, we compared the adverse health effects induced by PM-samples collected from new (woodchip boiler) and old (logwood boiler) technology heating appliances. Particulate samples (Dp<1μm) were collected using a Dekati Gravimetric Impactor (DGI). Healthy C57BL/6J mice were intratracheally exposed with equivalent masses of PM at multiple doses (1, 3, 10 and 15 mg/kg). At 4h and 18h hours after exposure, the lungs were lavaged and the bronchoalveolar lavage fluid (BALF) was assayed for indicators of inflammation and tissue damage (lactate dehydrogenase (LDH), total protein and total cell number). Overall, LDH and total protein concentrations and cell number in lungs of the mice were only slightly increased after 4 and 18 hours exposure to woodchip boiler and logwood PM compared to blank filter sample. There were no clear dose response relations in these parameters. Per same mass unit the particles from new technology boiler were slightly more potent inducers of these toxicological responses. However, with new technology the particulate emissions were significantly reduced.

PS 672 COMBUSTION CONDITIONS AFFECT TOXICOLOGICAL AND CHEMICAL PROPERTIES OF WOOD COMBUSTION PARTICLES.

M. Tapanainen[1], P. Jalava[1, 2], H. Lamberg[2], P. Hakulinen[1], P. Yli-Pirilä[2], J. Ruusunen[2], J. Mäki-Paakkanen[1], R. Hillamo[3], J. Jokiniemi[2, 4] and M. Hirvonen[1, 2]. *[1]National Institute for Health and Welfare, Kuopio, Finland, [2]University of Eastern Finland, Kuopio, Finland, [3]Finnish Meteorological Institute, Helsinki, Finland and [4]VTT Technical Research Centre of Finland, Espoo, Finland.* Sponsor: M. Viluksela.

Residential wood combustion influences local air quality by emitting large quantities of harmful fine particulate matter that may exacerbate respiratory symptoms and increase hospital admissions of cardiorespiratory patients. Previously published studies show that combustion condition, fuel and operational practices affect the particulate emissions. Nonetheless, toxicological characteristics of particles from divergent wood combustion processes are not well understood. We investigated the effect of wood combustion conditions on toxicological and chemical properties of fine particulate (Dp < 1μm) mass emitted from six different small-scale appliances. Production of inflammatory mediators MIP-2 and TNFα (ELISA immunoassay), cytotoxicity (MTT test, DNA content analysis) as well as genotoxicity (Comet assay) were measured after 24 hour exposure of mouse RAW264.7 cell line to the particulate samples. Genotoxic potential of the particles was assayed also in human BEAS-2B bronchial epithelial cell line. Mass spectrometry and ion chromatography systems were used to analyze chemical content (PAHs, inorganic ions and elements) of the particles. Combustion conditions had a significant impact on toxicity and chemical composition of particles, and human bronchial epithelial cell line proved to be more prone to genotoxic effects of particles than mouse macrophage cell line. Interestingly, inflammatory activities of all wood combustion samples were relatively low. Instead, particulate samples from incomplete combustion induced 2-fold increase in cell death and 3-fold increase in genotoxicity compared to those from more complete combustion. This was associated with high concentrations of PAHs and other organic compounds in these samples. Thus, adverse effects of wood combustion particles can be reduced by using proper technology and operational practices in residential heating.

PS 673 DENDRITIC CELLS IN ALLERGIC ASTHMA EXHIBIT HEIGHTENED FUNCTIONAL INFLAMMATORY RESPONSES ON ACTIVATION BY AMBIENT PARTICULATE MATTER.

M. A. Williams[1], S. M. Bauer[2] and S. N. Georas[2]. *[1]NHEERL - EPHD, U.S. - EPA, Research Triangle Park, NC and [2]Department of Medicine, University of Rochester, Rochester, NC.*

Rationale: Allergic asthma is partly defined by skewed Th2-type immunity in which dendritic cells (DC) and respirable ambient particulate matter (APM) may play important roles. This study has delineated functional differences in allergic asthmatic as compared to normal DC on activation by APM. Methods: We recruited 25 subjects (10 healthy controls; 9 house-dust mite allergic asthmatics; 6 non-atopic asthmatics) in an RSRB-approved study and generated DC from purified CD14+ monocytes. DC function was explored on activation by APM by measuring antigen endocytosis, cell-surface marker expression by flow cytometry, cytokine secretion by multiplex array, and stimulation of alloreactive naive CD4+ T cells including Th1/ Th2 responses. Mechanisms of DC activation by APM were explored by probing p38MAPK and p40/p42-ERK I/II signal pathways.
Results: Allergic asthmatic DC exhibited prolific endocytosis as compared normal DC ($p<0.05$) where APM dampened endocytosis ($p<0.05$) and expression of the endocytic receptor CD206, while CD83 was enhanced - concordant with normal DC maturation. By contrast, allergic asthmatic DC retained antigen uptake ($p<0.05$) and higher expression of CD206, CD83, the co-stimulatory molecules CD40, CD54, CD86 ($p<0.05$) and higher expression of MHC I and II ($p<0.02$), TLR2 and TLR4 as compared normals ($p=0.07$) - suggesting a non-canonical pathway of DC differentiation in allergic asthma. On activation by APM, DC from allergic asthmatics secreted inflammatory cytokines (TNFα, IL-6, IL-8, IL-10) that was at variance with normal DC. APM exposed asthmatic DC stimulated naïve CD4+ T cells to secrete greater levels of IL-13 as compared normal DC ($p<0.01$). APM also induced time-dependent phosphorylation of p38MAPK and ERK I/II and inhibiting those pathways markedly dampened APM-mediated DC activation and cytokine secretion.
Conclusion: Dysregulation of DC on activation by APM may have important consequences in the immune pathology of asthma and exacerbations of disease.

PS 674 CHEMOKINE-MEDIATED INDUCTION OF $CD11b^+Gr1^+$ MYELOID-DERIVED SUPPRESSOR CELLS (MDSCs) BY CANNABINOIDS AND THEIR ROLE IN IMMUNOSUPPRESSION.

V. L. Hegde, P. S. Nagarkatti and M. Nagarkatti. *Pathology, Microbiology, and Immunology, University of South Carolina, Columbia, SC.*

Cannabinoids from marijuana plant exhibit potent immunosuppressive properties, although precise mechanisms are unclear. MDSCs are a heterogeneous population of immature myeloid cells which suppress anti-tumor and inflammatory immune responses. Administration of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (10-50 mg/kg, *i.p.*) in BL/6 mice resulted in a dose-dependent induction of $CD11b^+Gr1^+$ cells in the peritoneum and spleen which peaked at around 16h. FACS-sorted $CD11b^+Gr1^+$ cells (>95%) induced by THC and CBD significantly suppressed T cell proliferation *in vitro* by polyclonal (ConA) as well as antigen-specific stimulation (ova-peptide in OT-II T cells), determined by thymidine incorporation. Adoptive transfer of these cells were able to suppress ConA-induced, T cell-mediated liver inflammation *in vivo.* Neutrophil controls induced by thioglycollate broth did not suppress T cell proliferation. ELISA for G-CSF and GM-CSF in sera and peritoneal exudates showed significant induction in G-CSF levels following THC and CBD injection, which peaked at 12-24h. Pre-treatment with anti-G-CSF in mice abrogated the accumulation of MDSCs induced by cannabinoids. THC and CBD-induced MDSCs consisted of $CD11b^+Ly6G^+Ly6C^{int}$ granulocytic and $CD11b^+Ly6G^{low}Ly6C^{high}$ monocytic subsets. THC induced MDSCs in a CB1 and CB2 receptor-dependent manner whereas, CBD in a TRPV1 (vanilloid) receptor–independent but PPAR-γ-dependent manner. Profiling studies for 609 mouse micro RNAs from Sanger database using total RNA from purified cells showed that THC and CBD-induced MDSCs exhibit unique miRNA expression profile when compared to bone marrow and spleen MDSCs, macrophages and dendritic cells from naïve mice. Our studies show that cannabinoids induce unique $CD11b^+Gr1^+$ MDSCs by chemokine-mediated mechanism which are highly immunosuppressive *in vitro* and *in vivo.* Induction of MDSCs appears to be an important mechanism of immunosuppression by cannabinoids (Supported in part by NIH grants R01ES09098, P01AT003961, R01ES019313).

PS 675 Δ⁹-TETRAHYDROCANNABINOL DECREASES ANTIGEN PRESENTATION ON DENDRITIC CELLS AFTER INFLUENZA INFECTION *IN VIVO* AND TOLL-LIKE RECEPTOR STIMULATION *IN VITRO.*

P. Karmaus[1, 3], W. Chen[2, 3], B. Kaplan[3, 4] and N. Kaminski[4, 3]. [1]*Cell & Molecular Biology Program, Michigan State University, East Lansing, MI,* [2]*Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI,* [3]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [4]*Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

Δ9-Tetrahydrocannabinol (Δ9-THC) alters the function of various cells of the immune system, in part, by exhibiting activity at two identified cannabinoid receptors, 1 (CB1) and 2 (CB2). Dendritic cells (DC) link the innate to the adaptive immune response and thus are viewed as the orchestrators of T cell responses. We employed an in vivo influenza A/PR8/34 (PR8) model and an in vitro bone marrow derived DC (bmDC) culture to assess the effects of Δ9-THC on DC function. To determine the contribution of CB1 and/or CB2 to modulation by Δ9-THC, we used mice devoid of CB1 and CB2 (CB1-/-CB2-/-). Three days post influenza challenge, lung infiltrating DC populations were determined by FACS for maturation (CD80 and CD86) and antigen presentation (MHC II). CD11b+CD11c+ conventional DC (cDC) were elevated in PR8 treated mice. Δ9-THC (75 mg/kg) reduced CD86 and MHC II surface expression on cDC in lungs from WT but not in CB1-/-CB2-/- mice. In vitro, CD11b+CD11c+ bmDC were generated and treated with LPS (1 μg/mL) and ssRNA (25 μg/mL), which induced expression of CD86 and MHC II. Compared to WT, bmDC from CB1-/-CB2-/- had greater basal and induced levels of CD86 and MHC II, yet both surface markers were reduced by Δ9-THC (10 μM) treatment in both genotypes. Δ9-THC affected neither CD80 expression nor viability. In addition, we tested the ability of bmDC to elicit T cell responses. While TLR agonists enhanced bmDC induced T cell proliferation and IFN-γ secretion, concurrent Δ9-THC treatment of bmDC suppressed both endpoints in a CB1/CB2-dependent manner. These studies suggest that DC are targets for Δ9-THC-mediated suppression and that CB1 and/or CB2 are important in regulating immune function and mediating Δ9-THC suppression of DC function. (Support: NIHDA07908)

PS 676 ANTI-INFLAMMATORY PROPERTIES OF ENDOGENOUS CANNABINOIDS CAN BE ATTRIBUTED TO INDUCTION OF A TRANSIENT POPULATION OF IMMUNOSUPPRESSIVE MYELOID DERIVED SUPPRESSOR CELLS THAT PROGRESS INTO IMMATURE MACROPHAGES.

A. R. Jackson, V. Hegde, P. Nagarkatti and M. Nagarkatti. *Pathology, Microbiology, and Immunology, University of South Carolina, Columbia, SC.*

Cannabinoids are a group of compounds that mediate their physiological and behavioral effects by activating specific cannabinoid receptors. Cannabinoid receptor 1 (CB1) is primarily expressed in the CNS. In contrast, cannabinoid receptor 2 (CB2) is predominantly expressed on immune cells. In addition to the exogenous cannabinoids found in the Cannabis plant, there are also endogenous cannabinoids (endocannabinoids, ECs), such as 2-arachadonoyl glycerol(2-AG) and N-arachidonoyl-ethanolamine(anadamide,AEA). The ECs also mediate their effects by activating CB1 and CB2 receptors. Cannabinoids have been shown to act as potent immunosuppressive agents and are used to mediate beneficial effects in a wide range of inflammatory diseases. Previously we have shown that ECs induce Myeloid Derived Suppressor Cells (MDSCs) that are CD11b+Gr-1+after a single i.p. administration. In the current study, we further characterized these cells by functional and expression profile. Functionally, these cells showed the ability to inhibit the T-cell proliferative response. Upon further examination, the response was shown to be dependent on the expression and function of Arginase-1. The mechanism of induction of these cells was linked to CB1 signaling as shown by experiments with knockout mice and pharmacological blocking. While the immunosuppressive MDSCs were induced within 12 hours post-EC injection, it was noted that on day 4, there was an increase in the proportion of cells that expressed Gr1-/Cd11b Lo/F480 Lo, thereby suggesting their differentiation towards myeloid pathway. Together, these experiments demonstrate that the anti-inflammatory properties of ECs can be explained, at least in part, through induction of immunosuppressive MDSCs (Supported in part by NIH grants R01ES09098, P01AT003961, R01ES019313).

PS 677 Δ⁹-TETRAHYDROCANNABINOL MODULATES ELICITATION AND FUNCTION OF CYTOTOXIC T LYMPHOCYTE (CTL) DIRECTED AGAINST HIV GP120.

W. Chen[1, 4], S. Pike[4], P. Karmaus[2, 4], B. Kaplan[4, 3] and N. Kaminski[3, 4]. [1]*Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI,* [2]*Cell & Molecular Biology, Michigan State University, East Lansing, MI,* [3]*Pharmacology & Toxicology, Michigan State University, East Lansing, MI and* [4]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI.*

Twenty-five percent of HIV patients use marijuana for appetite stimulation to attenuate HIV-associated nausea and wasting syndrome. The effects of cannabinoids on the immune systems of immunocompromised HIV patients are not well understood. Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) is the predominant psychoactive compound in marijuana, and is known to modulate immune competence. Depending on the magnitude of T cell activation, both positive and negative effects of Δ⁹-THC on T cell functions, e.g. IL-2 production, have been observed. A surrogate *in vitro* mouse model for presenting HIV gp120 antigens to T cells has been established to study the effects of Δ⁹-THC on elicitation and function of antigen-specific CTL. A gp120-expressing dendritic cell clone, $DC2.4_{gp120}$ C1, was generated to serve as antigen presenting cells (APC) to elicit gp120-specific T cells. $CD8^+$ T cell activation, shown by up-regulation of an early T cell activation marker, CD69, at 6 hr post the initiation of elicitation, and proliferation were observed. On day 5, CTL were restimulated with gp120-expressing target cells, $EL4_{gp120}$ C135. CTL activity was assessed by intracellular IFNγ staining. Higher percentages of $IFN\gamma^+CD8^+$ cells were detected when $EL4_{gp120}$ C135 was used as target cells compared to parental EL4, suggesting a gp120-specific response. Under current activation conditions, induced IFNγ secretion was enhanced by Δ⁹-THC when added during the elicitation phase. CD69 expression and proliferation of $CD8^+$ T cells were also enhanced by Δ⁹-THC, suggesting an effect on the early stage of T cell activation. Collectively, these results indicate that $DC2.4_{gp120}$ C1 cells are capable of eliciting a gp120-specific CTL response, and Δ⁹-THC can enhance the elicitation and function of gp120-specific CTL. (Supported by NIH DA07908)

PS 678 EFFECTS OF CANNABINOIDS ON ANTI-HIV-TAT INDUCED ANTIBODY PRODUCTION.

E. Velez[1, 3], R. Dasgupta[1], N. E. Kaminski[1, 2] and B. F. Kaplan[1, 2]. [1]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI,* [2]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI and* [3]*Chemistry, University of Puerto Rico at Cayey, Cayey.*

Late stage Human Immunodeficiency Virus-1 (HIV) infection is characterized by an immune response insufficiency, which manifests itself as susceptibility to infection by the occurrence of, among others, Kaposi's sarcoma or B-cell lymphoma. The immune response elicited by several HIV proteins has been studied. Among these is Tat, an HIV protein that facilitates and accelerates the transcription of most of the HIV genome. Since many people infected with HIV use marijuana for appetite stimulation, and marijuana compounds, such as Δ9-tetrahydrocannabinol (Δ9-THC), are often immunosuppressive, there is an interest in determining the effect of marijuana compounds on initial anti-HIV immune responses. The objectives of the studies are to determine if Tat-induced antibody production is affected by cannabinoid compounds. A dendritic cell line (DC2.4) was engineered to express Tat (DC2.4-Tat) as a surrogate for HIV-infected antigen presenting cell. The DC2.4-Tat bulk culture was cloned in limiting dilution and several clones expressed high levels of Tat and the puromycin resistance gene. Expression of Tat in DC2.4 cells increased cell size and levels of the activation markers, MHC class I, MHC class II, CD80 and CD86. DC2.4 or DC2.4-Tat was used to stimulate IgM antibody from splenic B cells. We demonstrated that DC2.4-Tat cells specifically induced IgM at 7 days and that pre-treating DC2.4-Tat with LPS boosted that response. Moreover, Δ9-THC pre-treatment of SPLC reduced IgM production at 7 days. Overall, these results suggest that co-incident marijuana use at the time of initial HIV infection might suppress early anti-HIV immune responses. (Supported in part by NIH RO1DA07908, R25NS065777, and the 2010 Michigan State University Summer Research Opportunity Program).

PS 679 GLOBAL AND TEMPORAL GENE EXPRESSION ANALYSIS OF PERITONEAL MACROPHAGES IN A MOUSE ALCOHOL BINGE DRINKING MODEL WITH REDUCED RESISTANCE TO SEPSIS.

X. Deng[1], B. Cheng[2], R. Fan[2] and S. Pruett[1]. [1]*Department of Basic Sciences, Mississippi State University, Mississippi State, MS and* [2]*Cellular Biology and Anatomy, Louisiana State University Health Sciences Center, Shreveport, LA.*

Acute ethanol consumption has been found to be a significant risk factor for mortality in patients with sepsis. The binge drinking model in mice developed by our laboratory has produced similar findings. At earlier time (1-2 hr) in our mouse

model, ethanol was found to inhibit the innate immune response such as cytokine productions in this model. The ethanol-treated mice sustained more bacteria growth in their peritoneal cavity as compared to control septic mice. Much more mortality occurs 18 hrs after E. coli challenge in the ethanol-treated mice. However, the exact cellular and molecular mechanisms of such inhibition of innate immune responses and the more lethality later are largely unknown. Thus, a microarray study examining the global gene expression profiles of the peritoneal immune cells at various time (1 hr, 2 hr and 18 hr) after E. coli challenge with or without ethanol pretreatment was conducted. The data confirms that ethanol inhibits early innate immune response to E. coli, including TLR signaling, cytokine/chemokines production, macrophage mobilization and bactericidal activity. Functional assay suggests that ethanol inhibits the leukocytes activation and the production of bactericidal products (i. e., nitric oxide) rather than inhibits leukocytes accumulation in the site of inflammation. This early inhibition leads to bacteria overgrowth and maybe macrophage "hyper-reactive" stage at later time (18 hr). In addition, the gene expression data here suggests novel molecular pathways, which may contribute to the inhibition of the immune response, such as inhibition of an interferon amplification loop, protein translation initiation, phosphlipase signaling and CDC42 activation. This work was supported by NIH grant R01AA09595 from NIAAA.

PS 680 METAL OXIDE NANOPARTICLES MODULATE EXOSOMES.

B. Andersson[1], T. Bürki-Thurnherr[2], H. Krug[2], B. Fadeel[3], A. Scheynius[1] and S. Gabrielsson[1]. *[1]Clinical Allergy Research Unit, Karolinska Institutet, Stockholm, Sweden, [2]Federal Laboratories for Material Science and Technology, St. Gallen, Switzerland and [3]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

Metal oxide nanoparticles (MOnp) are widely used in the paint and coating industry as well as in cosmetics, and they have been extensively studied from a toxicology point of view. However, the interactions of MOnp with the immune system have not yet been fully addressed. Dendritic cells (DC) are highly efficient antigen presenting cells and are known to secrete membrane vesicles in the nano range (50-100 nm) referred to as exosomes. Exosomes have profound functions involving cell to cell communication, delivery vehicles, and immune system activators or suppressors. We hypothesise that MOnp can interact with DC and influence the exosome production and function. Therefore, we exposed human monocyte-derived DC (MDDC) to two commonly used and commercially available MOnp; TiO2 and ZnO. MDDC viability was not affected by TiO2, whereas ZnO induced a dose dependent increase in cell death (AnnexinV/PI staining) and caspase activity (CaspAce). Non-toxic exposure of MOnp did not induce changes in DC surface marker expression. The culture supernatant was collected and via several ultra centrifugation steps, exosomes were isolated. Exosomes were coated on anti-MHC-II conjugated Dynabeads® and subjected to TEM analysis and flow cytometry. TEM images revealed that nanoparticles were not found inside the exosomes. Exosomes from MDDC exposed to ZnO exhibited a tendency of decreased expression of the tetraspanin CD81, the co-stimulatory marker CD40, and the antigen presenting complex MHC-II. ZnO also induced a slight increase in apoptosis inducing FasL expression, whereas TiO2 induced a decrease of FasL expression, compared to negative control exposure. We conclude that exposure of MDDC to MOnp can influence the exosome phenotype which might provoke differences in immune cell regulation and cell death. Sponsored by the Seventh Framework Program of the European Commission (FP7-NANOMMUNE, grant no. 214281).

PS 681 ROLE OF NEUTROPHILS IN A MOUSE MODEL OF CHEMICAL-INDUCED ASTHMA.

S. Smulders[1], V. De Vooght[1], G. Opdenakker[2], B. Nemery[1], P. Hoet[1] and J. Vanoirbeek[1]. *[1]Research Unit for Lung Toxicology, K.U. Leuven, Leuven, Belgium and [2]Rega Institute for Medical Research, Laboratory of Immunobiology, K.U. Leuven, Leuven, Belgium.*

Asthma is often characterized by a predominant neutrophilic inflammation. In this study we tried to determine the specific role of neutrophils in the airway hyperreactivity (AHR) and lung inflammation in a mouse model of chemical-induced asthma by depleting the mice from neutrophils before pulmonary challenge. On days 1 and 8, BALB/c received 0.3% toluene diisocyanate (TDI) or vehicle (acetone olive oil) on each ear, followed by 2 intraperitoneal injections of cyclophosphamide (CP) or saline on days 11 and 13, or 1 intravenous injection of anti-GR1 antibody (aGR1) or HBSS on day 13. On day 15, they received an oropharyngeal challenge of 0.01% TDI or vehicle. On day 16, AHR to methacholine was measured. Bronchoalveolar lavage differential cell count and total serum IgE were assessed. Total lymphocyte count, lymphocyte subpopulations (CD4, CD8, CD25 and CD19) and release of IL4, IL10, IL13 and IFNγ by lymphocytes of the auricular lymph nodes were assessed. CP and aGR1 induced a full neutrophil depletion. CP led to a complete disappearance of the AHR upon methacholine provocation, while aGR1 caused only a partial decrease in AHR. Furthermore, a full eosinophil depletion was observed after CP- and aGR1-injections. CP-injected TDI-mice showed an almost full depletion of the number of auricular lymphocytes, while this was only slightly decreased in the aGR1-injected TDI-mice, mainly caused by depletion of cytotoxic T-cells. A decrease of the concentration of IL-13 was detected in the aGR1-injected TDI-mice compared to the HBSS-injected TDI-mice. In CP-injected TDI-mice, decreased total serum IgE was observed compared to the saline-injected TDI-mice. In conclusion, CP and aGR1 are potent neutrophil depletion agents, which clearly influence AHR in our mouse model of chemical-induced asthma, but due to side effects of the treatment on other cell-types the neutrophil specific role could not yet be clarified. IAP: P6/35

PS 682 EFFECTS OF *PENICILLIUM* MYCOTOXINS ON BOVINE MACROPHAGES *IN VITRO.*

H. J. Boermans[2], S. Oh[1], N. A. Karrow[1], B. Sharma[1] and S. Haladi[3]. *[1]Animal and Poultry Sciences, University of Guelph, Guelph, ON, Canada, [2]Biomedical Sciences, University of Guelph, Guelph, ON, Canada and [3]Alltech, Guelph, ON, Canada.*

Penicillium fungal mycotoxins are often found in grains, crops, fruits, and fermented products, especially during pre- and post- harvest, as well as storage periods. Many studies have assessed the impact of oral exposure to mycotoxins from Fusarium and Aspergillus species on livestock as well as human health. However, little is known about the impact of Penicillium mycotoxins on these aspects, especially their potential immunotoxicity. In this study, the potential immunotoxicity of citrinin (CIT), ochratoxin A (OTA), patulin (PAT), mycophenolic acid (MPA) and penicillic acid (PA) was evaluated using a bovine macrophage cell line (BOMAC) by first assessing their potential cytotoxicity, and then their effects on cell proliferation. BOMAC cells were exposed to a range of mycotoxin concentrations, and then to different mycotoxin combinations for 48 hrs. Some cytotoxicity was evident at concentrations greater than 2.4 uM for PAT, and 160 uM for PA, however, at the IC50 (concentration that inhibits 50% cell proliferation) for these mycotoxins, no cytotoxicity was observed. The mycotoxin IC50s from most potent to least potent were 0.56 uM (PAT), 12.88 uM (OTA), 29.85 uM (PA), and 91.20 uM (CIT). Concentrations of MAP greater than 80 uM did not inhibit cell proliferation enough to calculate an IC50. Ten different combinations of mycotoxins were tested at concentrations equivalent to their IC25 without any cytotoxicity, and proliferation was significantly reduced with the following seven mycotoxin combinations: CITxOTA, CITxMPA, CITxPA, OTAxPAT, OTAxMPA, OTAxPA, MPAxPA.

PS 683 EFFECT OF ASBESTOS EXPOSURE ON PRODUCTION OF CYTOKINES, APOPTOSIS, AND CELL PROLIFERATION RELATED WITH DIFFERENTIATION OF HUMAN CTL.

N. Kumagai, Y. Nishimura, M. Maeda, H. Hayashi and T. Otsuki. *Hygiene, Kawasaki Medical School, Kurashiki, Japan.*

Asbestos exposure can cause malignant mesothelioma and lung cancer. In the antitumor immunity, cytotoxic T lymphocytes (CTL) play a critical role. Previously, we reported that asbestos exposure suppressed the induction of human CTL during mixed lymphocyte reactions (MLR), accompanied with the decreases in IFN-γ and TNF-α but not IL-2. However, the time of assay for TNF-α and IL-2, day 7 after MLR, might not be optimal, because of no difference between control and allogenic conditions. The present study examined the kinetics of TNF-α and IL-2 production during 7-days MLR upon asbestos exposure, by using ELISA. In addition, the proliferation and apoptosis of $CD8^+$ cells were also examined by flow cytometry. Human peripheral blood mononuclear cells (PBMCs) were cultured with irradiated allogenic PBMCs with or without chrysotile B (CB) asbestos at 5 μg/ml. The production of TNF-α did not differ between control and allogenic conditions at day 2 and 4 as well as day 7 after MLR. However, the decrease in production of TNF-α upon CB exposure was observed only at day 7, but not at day 2 or 4. The production of IL-2, which had a peak at day 4 after MLR and no difference between control and stimulating cultures, did not decrease upon CB exposure during the entire period of culture. The percentage of annexin V^+ apoptotic cells in $CD8^+$ cells stimulated allogenically did not increase upon CB exposure. Additionally, the percentage of $CD25^+$ cells and $CD45RO^+$ cells in apoptotic $CD8^+$ cells decreased upon CB exposure. In contrast, CB exposure suppressed the increase in CFSE-negative $CD8^+$ cells, caused by cell division, upon allogenic stimulation. Thus, taken together with our previous reports, these results indicate that CB exposure suppresses the induction of CTL, accompanied with decreased productions of TNF-α and IFN-γ and suppressed proliferation of $CD8^+$ cells independent of IL-2 production. These suppressive effects of asbestos exposure on induction of CTL might promote the generation of tumor disease.

PS 684 1, 3-DIBROMOPROPANE UP-REGULATES INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION *VIA* NF-KB ACTIVATION IN MACROPHAGES.

J. Yang[1], E. Han[1], J. Im[1], K. Lee[2], T. Jeong[3] and H. Jeong[1]. [1]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea,* [2]*Pharmacy, Chonnam National University, Gwangju, Republic of Korea and* [3]*Pharmacy, Yeungnam University, Kyungsan, Republic of Korea.*

Bromopropane has been used in the workplace as an alternative to ozone-depleting solvents. In this study we investigated the effect of 1,3-dibromopropane (DBP) on nitric oxide (NO) and proinflammatory cytokines production in mouse macrophages. DBP induced the production of NO and proinflammatory cytokines such as IL-1, IL-6 and TNF- and expression levels of these genes in a dose-dependent manner. The NF-κB sites were identified in the promoter of the inducible NO synthases (iNOS) and proinflammatory cytokines genes. Transient transfection revealed that NF-kB mediated the DBP-induced increase in the iNOS and proinflammatory cytokines expression levels. This demonstrates that DBP stimulates macrophage activation via NF-kB transactivation. These results suggest that DBP has the potential to be inflammatory and that is has previously unrecognized immunomodulating activity.

PS 685 SYNERGISM OF LEPTIN SIGNALING AND POST TRANSLATIONAL PROTEIN OXIDATION IN BROMODICHLOROMETHANE EXPOSURE IS KEY TO THE DEVELOPMENT OF STEATOHEPATITIS OF OBESITY.

S. Chatterjee, J. Clark, M. Kadiiska and R. P. Mason. *Laboratory of Toxicology and Pharmacology, NIEHS, Research Triangle Park, NC.*

Disinfection byproducts like bromodichloromethane (BDCM) at a dose of at least 5 mMoles/kg is known to be hepatotoxic with necroinflammatory lesions. Although direct exposure to high doses of BDCM is rare, low exposures from the environment are not uncommon. These doses are well tolerated by normal healthy individuals but can be potential risk factors for inflammatory liver injury like steatohepatitis in obese subjects. We tested the hypothesis that metabolic oxidative stress arising from such low exposures in obese subjects synergise with high leptin levels and lead to exacerbation of steatohepatitis through lipid peroxidation, protein oxidations and innate immunity. Results indicated that BDCM exposure (2mMoles/kg) caused significantly increased lipid peroxidation in hepatocytes of diet-induced obese (DIO) and leptin-k/o mice at 6h. There was also a significant increase in protein radical adducts and post-translational tyrosine nitration in sinusoidal cells including Kupffer cells at 24 hrs when compared to leptin-k/o or lean control mice at 24h. Confocal microscopy studies and liver histopathology from wild-type DIO obese mice showed perivenular aggregation of Kupffer cells and marked necroinflammation. The role of CYP2E1, one of the enzymes of the cytoctochrome P450 family in BDCM bioactivation, and NADPH oxidase were also confirmed using specific inhibitors for these enzymes. Results also showed that Kupffer cells from DIO mice exposed to BDCM had enhanced TNF-alpha release when compared to corresponding lean controls and leptin-knockout mice. Kupffer cells co-incubated either with 4-hydroxy Tempol, a superoxide dismutase mimetic, or higher concentrations of the spin trap DMPO (100mM) significantly reduced macrophage activation. Taken together, our data for the first time suggest that BDCM bioactivation from the environmental sources and leptin generates an inflammatory cascade and free radical damage in obese individuals.

PS 686 EFFECT OF LEAD EXPOSURE ON THE MACROPHAGES ACTIVATION THROUGH TLR4.

A. L. Luna[1, 2], N. A. Torres-Aviles[1], L. C. Acosta-Saavedra[1], E. K. Silbergeld[3] and E. S. Calderon-Aranda[1]. [1]*Department Toxicologia, Cinvestav, Mexico, Mexico,* [2]*Biomedicina Molecular, ENMH-IPN, Mexico, Mexico and* [3]*BSPH, Johns Hopkins University, Baltimore, MD.*

Lead is a metal that increases susceptibility to infection by pathogens such as Escherichia coli and Staphylococcus aureus. Macrophages recognized PAMP's (patterns of molecules associated with pathogens) through TLR (Toll Like Receptors). The binding of PAMP's by TLR induce macrophages activation, production of bactericidal species like nitric oxide (NO•-), and the secretion of inflammatory cytokines. The aim was to study the effect of environmentally relevant non-cytotoxic lead concentrations, on the activation of macrophages through TLR4, using two lipopolysaccharides (LPS) which are derived from different pathogens to determine if the effect of Pb is dependent of the pathogen. Methods: murine macrophages J774A.1 were exposed to lead acetate (AcPb) (range from 0.005 to 250μg/dL) and activated with lipopolysaccharide (LPS) from Salmonella typhimurium or Escherichia coli under two schemes: a) Pre-exposure to AcPb and subsequent activation, b) Co-exposure to AcPb and LPS. Viability was assessed by the MTT method, and macrophage activation by NO•- production (Griess method), and secretion of IL-1β, IL-6 and TNF-α (ELISA). Results: 0.05 to 25μg/dL of AcPb do not affect viability and were to all assays. The pre-exposure to AcPb decreases NO•- induced with E. coli but does not with Salmonella; in the co-exposure AcPb increases the NO•- induced by E. coli but does not in the NO•- induced with Salmonella. The pre-exposure to AcPb increases IL-1β induced with both LPS, decreases IL-6 induced with E. coli but does not IL-6 induced with Salmonella. AcPb decreases TNF-α induced with E. coli and increases this cytokine only at 5μg/dL of AcPb. In co-exposure scheme AcPb decreases IL-1β induced with both LPS, decreases IL-6 and TNF-α with both LPS. The results suggest that AcPb affect macrophage activation dependent of PAMP's origin and probably alteration of TLR4 pathway at extracellular level.

PS 687 CONTINUOUS AND LOW-DOSE EXPOSURE TO ASBESTOS ENHANCES TGF-β1 PRODUCTION IN A HUMAN ADULT T CELL LEUKEMIA VIRUS-IMMORTALIZED T CELL LINE, MT-2.

T. Otsuki, M. Maeda, N. Kumagai, N. Miyahara, M. Katoh, S. Yamamoto, T. Hatayama and Y. Nishimura. *Hygiene, Kawasaki Medical School, Kurashiki, Japan.*

Asbestos exposure not only causes pulmonary fibrosis, but also induces various tumors such as lung cancer and malignant mesothelioma. To elucidate the immunological alteration in asbestos-related tumors, an asbestos-induced apoptosis-resistant subline (MT-2Rst) was established from a human adult T cell leukemia virus-immortalized T cell line (MT-2Org) by long-term, low-level exposure to asbestos chrysotile-B (CB). The mechanisms of acquisition of resistance against the asbestos-induced apptosis are activation of Src-family kinases, overexpression and secretion of IL-10, phosphorylation of STAT3, and activation of Bcl-2. In this study, transforming growth factor-beta1 (TGF-β1) knockdown using lentiviral vector-mediated RNA interference showed that MT-2Rst cells secrete increased level of TGF-β1 as well as IL-10, and acquire resistance to TGF-β1-mediated growth inhibition via the deficiency in TGF-β1/SMAD signaling. However, Annexin V assay indicated that TGF-β1 resistance in MT-2Rst cells were not directly involved in the acquisition of resistance to apoptosis that is triggered by CB exposure. Alternatively, we showed that TGF-β1 production in MT-2Org cells is mediated through the activation of the mitogen-activated protein kinases (MAPKs), ERK1/2 and p38, by exposure to CB. Furthermore, TGF-β1-knockdown cells and treatment with MAPKs inhibitors revealed that MT-2Rst cells secrete high level of TGF-β1 via mainly phospholylation of p38. Taken together, long-term, low-level exposure to CB on MT-2Org cells induce regulatory T cells-like phenotype, suggesting that chronic exposure to asbestos lead to a state of immune suppression.

PS 688 EFFECT OF *VIBRIO VULNIFICUS* LIPOPOLYSACCHARIDE (LPS) ON RAT BRAIN MICROGLIA: CYTOKINE AND CHEMOKINE EXPRESSION PROFILING BY ANTIBODY ARRAY TECHNOLOGY.

L. D. Ottenhoff[1], N. Patel[1], M. L. Hall[1], K. B. Glaser[2, 1], P. B. Jacobson[2], D. Rowley[3], C. De Castro[4], A. Molinaro[4] and A. M. Mayer[1]. [1]*Pharmacology, Midwestern University, Downers Grove, IL,* [2]*Abbott Laboratories, Chicago, IL,* [3]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI and* [4]*Chimica Organica e Biochimica, Università di Napoli Federico II, Naples, Italy.*

We have reported that *Vibrio vulnificus* LPS (VvLPS)-stimulated rat neonatal brain microglia (BMG) release of TNF-α, TXB_2, O_2^- and MMP-9 (The Faseb Journal 21(5): A 404, 2007), and interleukin-1 α (IL-1α), IL-6, and MIP-2/CXCL2 (The Toxicologist, 108 (S1): 12, 2009). The purpose of this investigation was to determine whether additional cytokines and chemokines were present in conditioned media of BMG cultures treated with VvLPS, using the RayBio® biotin label-based rat antibody array technology (RayBiotech, Inc., Norcross, GA). VvLPS was isolated from VvLPS strain MO6-24/O by hot phenol/water extraction. Neonatal rat BMG were treated *in vitro* with (1 x 10^3 ng/mL) VvLPS for 17 h. The study revealed the presence of several upregulated cytokines and chemokines in conditioned media (greater than two-fold increase vs. unstimulated BMG), namely: IL-6 (10), MCP-1/CCL2 (6.5), MIP-1α /CCL3 (5.6), BDNF(3.1), and Cinc-2α/β/CXCL3 (1.3); and one downregulated cytokine (greater than two-fold decrease vs. unstimulated BMG): IL-1α (-2.8). We conclude that the RayBio® protein array technology has enabled detection of additional cytokines and chemokines in conditioned media from 17 h *in vitro* VvLPS-treated BMG. Confirmation of RayBio® protein array results with chemokine and cytokine-specific ELISAs is currently ongoing in our laboratory. Our results provide further insight into proinflammatory mediators released by VvLPS–treated BMG that may contribute to self-injury and/or neuronal toxicity. Financial support by Midwestern University is acknowledged.

PS 689 ENHANCED SUPPRESSIVE FUNCTION ON REGULATORY T CELL-LIKE CELL LINE, MT-2, BY CONTINUOUS EXPOSURE TO ASBESTOS.

M. Maeda[1], Y. Chen[1], Y. Nishimura[1], N. Kumagai[1], H. Hayashi[1], Y. Miura[2] and T. Otsuki[1]. *[1]Hygiene, Kawasaki Medical School, Kurashiki, Okayama, Japan and [2]Molecular Genetics, Kyushu University, Fukuoka, Japan.*

[Introduction] Chronic exposure to asbestos occurs malignant mesothelioma. As this disease has a long incubation period, $CD4^+CD25^+$ regulatory T (Treg) cells-mediated anti-tumor immune response may be impaired. To reveal whether Treg cells exposed to asbestos attenuate an anti-tumor immune function in malignant mesothelioma patients, six chrysotile-induced apoptosis-resistant sublines (MT-2Rsts) were established by long-term (more than 8 months) and low-level (10 μg/ml) exposure to chrysotile-A or -B from the HTLV-1-immortalized human T-cell line MT-2 (MT-2Org) having a Treg-like suppressive function, and their suppressive functions were estimated by mixed lymphocyte reaction (MLR). [Methods] The regulatory function in an allogeneic MLR was determined using [^{3}H]-Thymidine incorporation. Expression of Treg markers (Foxp3, CTLA-4 and GITR) was analyzed by flow cytometry. The mechanisms of the suppressive function were clarified in MT-2Rst using a CFSE assay. Involvement of immunosuppressive cytokines IL-10 and TGF-β1 in suppressive function was investigated using an shRNA knockdown system. [Results and Discussions] Five MT-2Rst cell lines suppressed proliferation of $CD4^+CD25^-$ responder T-cells (Tresp) upon stimulation with irradiated allogeneic PBMCs more strongly than MT-2Org. The results of flow cytometry showed that MT-2Org and MT-2Rsts cells expressed high levels of Foxp3 and GITR. When Tresp were stimulated with coated anti-CD3 antibody and autologous induced-dendritic cells, the proliferation was suppressed by MT-2Rst more strongly than MT-2Org. Furthermore, shRNA knockdown showed that IL-10 and TGF-β1 produced from MT-2Rst suppressed the proliferation of Tresp. In conclusion, these findings revealed that chronic exposure to asbestos enhanced the suppressive function through cell contact-dependent mechanisms and soluble factors in MT-2Rst, suggesting that asbestos exposure enhances Treg suppressive function and lead to a decrease in anti-tumor immune function.

PS 690 HISTONE DEACETYLASES PLAY A CRITICAL ROLE IN REGULATING IFNα-MEDIATED ANTI-HCV GENE EXPRESSION.

S. Mathews[1], S. Joshi-Barve[1], C. McClain[1, 2] and S. Barve[1]. *[1]Department of Medicine/GI, University of Louisville, Louisville, KY and [2]Robley Rex Veterans Administration Medical Center, Louisville, KY.*

Hepatitis C virus (HCV) is a major cause of chronic liver disease and is a huge burden on the United States healthcare system. The FDA-approved standard of care is pegylated interferon-alpha (IFNα) in combination with ribavirin; however up to 50% of patients do not respond to this therapy. Understanding the regulatory mechanisms that play an essential role in the expression of IFNα anti-HCV genes may lead to the development of therapeutic options that increase the effectiveness of IFNα. The present work was carried out to examine the potential role of histone deacetylases (HDACs) in the regulation of IFNα-stimulated anti-HCV genes.

Two class I HDAC inhibitors (HDACi), trichostatin A (TSA) and suberic bishydroxamate (SBHA) were used to treat human hepatoma cells (Huh7 and HCVR; Huh7 stably transfected with an HCV replicon) or primary human hepatocytes prior to stimulation with IFNα. HDACi pretreatment inhibited induction of several IFN-mediated antiviral genes, as analyzed by real-time PCR. Importantly, this inhibition corresponded to decreased activation of the interferon stimulated response element (ISRE) found in the promoters of IFNα antiviral genes. Additionally, HDACi led to retention of phospho-STAT1 in the cytoplasm, potentially due to altered acetylation of STAT1, and corresponded to impaired expression of antiviral proteins. Immunoprecipitation analysis using lysates from IFNα-stimulated cells identified HDAC1 binding to STAT1 in the nucleus and this interaction was inhibited by HDACi. Since HDACi block the actions of class I HDACs, siRNA was used to specifically identify HDAC1 and HDAC3 as critical regulators of IFNα antiviral signaling. Taken together, the data from these experiments reveal a unique requirement for HDACs 1 and 3 in regulating IFNα-mediated anti-HCV gene expression in hepatocytes.

PS 691 ZIRAM INDUCES APOPTOSIS IN HUMAN IMMUNE CELLS.

Q. Li, M. Kobayashi and T. Kawada. *Nippon Medical School, Tokyo, Japan.*

Background: Ziram as a dithiocarbamate fungicide is widely used throughout the world in agriculture and as an accelerating agent is used in latex production. It has been reported that ziram shows immunotoxicity.

Purpose: To explore the mechanism of ziram-induced immunotoxicity, we investigated ziram-induced apoptosis/necrosis and its underlying mechanism in human immune cells.

Materials and Methods: U937, a human monocyte like cell line and NK-92MI, a human NK cell line, were treated with ziram at 0.0312-2 μM for 2-24 h at 37°C in a 5% CO2 incubator. Apoptosis/necrosis induced by ziram was determined by analysis of FITC-Annexin-V/PI staining and the intracellular level of active caspase-3 by flow cytometry and DNA fragmentation analysis (1-3). Mitochondrial cytochrome c release in U937 cells induced by ziram was also determined by flow cytometry (3).

Results: We found that ziram induced apoptosis/necrosis in both U937 and NK-92MI cells in a dose- and time-dependent manner, as shown by FITC-Annexin-V/PI staining. DNA fragmentation was detected when U937 cells were treated with 0.5, 1 or 2 μM ziram for 24 h. Ziram also induced an increase of intracellular active caspase-3 in U937 and NK-92MI cells in a dose-dependent manner, and a caspase-3 inhibitor, Z-DEVD-FMK, significantly inhibited the ziram-induced apoptosis. Moreover, it was found that ziram induced mitochondrial cytochrome c release in U937 cells.

Conclusions: These findings indicate that ziram can induce apoptosis/necrosis in both U937 and NK-92MI cells, and this effect is partially mediated by activation of intracellular caspase-3 and mitochondrial cytochrome c release. From this perspective, we speculate that ziram-induced immunotoxicity may be mediated by apoptosis in immune cells.

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PS 692 ENHANCEMENT OF HUMORAL IMMUNITY BY THE ENDOCRINE DISRUPTER PROPANIL IS NOT ALTERED BY NEONATAL ANDROGEN TREATMENT.

R. Schafer[1], S. Hileman[2], R. Goodman[2] and I. Holaskova[1]. *[1]Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, WV and [2]Physiology and Pharmacology, West Virginia University, Morgantown, WV.*

Studies in our laboratory demonstrated that the pesticide propanil enhances the immune response to a vaccine that is dependent on the gonads in females but is independent of the gonads in males. This effect was independent of estrogen, progesterone and testosterone. The dramatically different response of gonadectomized male and female mice to propanil raised the possibility that the immune response is sexually differentiated and accounts for the sexually-dimorphic effect of propanil in adults. We hypothesized that androgen treatment of neonatal female mice would normalize the enhanced antibody response after propanil exposure in ovariectomized (OVX) adult females.

Neonatal C57Bl/6 pups were treated subcutaneously with testosterone propionate (TP, 400 μg in corn oil) or corn oil within 24 hours of birth. Efficacy of TP treatment in females was determined by vaginal smears and uterine weight. At 6 weeks of age female mice were OVX or sham OVX. Mice were vaccinated at 10 weeks of age with heat-killed *Streptococcus pneumoniae* and treated with propanil or vehicle. One week later the spleens were collected and the antibody response to the vaccine was determined by ELISPOT assay.

Testosterone treatment resulted in abrogation of the estrous cycle. Propanil significantly increased IgM and IgG antibody producing cells in males treated with TP at birth. In females given TP at birth, propanil significantly increased IgM, IgG, and IgG3 responses. Ovariectomy abrogated the increase demonstrating that the enhanced immune response after propanil exposure is still dependent on the ovaries in TP treated females. Thus, neonatal androgen treatment produced the expected effects on the reproductive system but did not alter the normal immune response to *S. pneumoniae* vaccine in adults. This indicates that the immune system of females was not masculinized during the neonatal time period and ovarian function is crucial for the enhanced response.

PS 693 ASBESTOS EXPOSURE CAUSES DECREASE IN PRODUCTION OF CYTOKINES RELATED TO NK CELL ACTIVATION.

Y. Nishimura, N. Kumagai, M. Maeda, H. Hayashi and T. Otsuki. *Hygiene, Kawasaki Medical School, Kurashiki, Japan.*

Recently, we reported that peripheral blood NK cells in patients with malignant mesothelioma (MM) showed low cytotoxicity with decrease in NKp46, one of receptors utilized to recognize targets and transduce activation signal, which also decreased in NK cells included in PBMCs cultured with chrysotile B (CB) asbestos. Additionally, purified NK cells showed an increase in NKp46 when co-cultured

with PBMCs without cell-cell contact using culture insert, and they showed a decrease in NKp46 when CB was added into the culture area of PBMCs. Therefore, we examined production of cytokines related to NK cell activation in the culture of PBMCs upon exposure to CB. PBMCs were cultured in the media supplemented with IL-2 upon exposure to CB at 5 μg/ml, and assayed for production of cytokines at secreted protein and mRNA levels by flow cytometry and real-time PCR. The analysis of culture supernatants showed decreases in TNF-α, IFN-γ and IL-12 in the culture of PBMCs with CB, whereas the amounts of IL-15 and IL-18 did not decrease. The four cell populations, monocyte-lineage cells (MLCs), defined by forward and side scatters, CD4+CD8β- T, CD4-CD8β+ T and CD4-CD8β-CD56+ NK cells were sorted from PBMCs cultured with or without CB. The MLCs showed a higher mRNA level of TNF-α than the other populations, and they showed the decrease in that mRNA level upon exposure to CB. The mRNA level of IFN-γ decreased in CD4+ T cells upon CB exposure, but it decreased a little in CD8+ T cells or did not in NK cells. In addition, the flow cytometric analysis of PBMCs showed the marked decreases in both populations of CD14+CD11c+ and CD14dim/-CD11c+ MLCs upon exposure to CB. These results indicate that CB exposure caused the decreased production of cytokines related to NK cell activation in the culture of PBMCs, accompanied with decrease in MLCs. The decreases in TNF-α, IFN-γ and IL-12 might be related with decreased cytotoxicity of NK cells in PBMCs cultured upon exposure to CB.

PS 694 IFNγ PRODUCTION BY RESTIMULATED NRF2-NULL T CELLS IS MODULATED BY PRESENCE OF WILD-TYPE ANTIGEN-PRESENTING CELLS.

C. E. Rockwell[1], P. E. Fields[2] and C. D. Klaassen[1]. [1]*Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS and* [2]*Department of Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS.*

Nrf2 is a transcription factor that upregulates cytoprotective genes in response to cell stress. Nrf2 also has anti-inflammatory effects in numerous models of inflammation. Because the T cell cytokine, IFNγ, promotes many types of inflammation, the purpose of the present studies was to determine the role of Nrf2 in IFNγ production by T cells. The Nrf2 activator, tBHQ, markedly suppresses IFNγ production by wild-type T cells activated with anti-CD3/anti-CD28, but not Nrf2-null T cells. Furthermore, Nrf2-null T cells produce markedly more IFNγ upon activation by anti-CD3/anti-CD28 than wild-type T cells. In addition to primary activation, the role of Nrf2 in regulating IFNγ production in T cells restimulated with anti-CD3 and anti-CD28 5 days after primary activation was also investigated. Similar to primary activation, Nrf2-null CD4+ T cells restimulated in this manner produce significantly more IFNγ than wild-type CD4+ cells. Interestingly, in the presence of irradiated wild-type antigen-presenting cells during primary activation, there was no difference in IFNγ production by restimulated CD4+ cells from wild-type and Nrf2-null mice. Collectively, the present studies demonstrate that Nrf2 negatively regulates IFNγ production in T cells activated with anti-CD3 and anti-CD28. In addition, these studies suggest that the presence of wild-type antigen-presenting cells can restore homeostatic IFNγ production in Nrf2-null T cells after secondary activation. Overall, these findings suggest a dysregulation in the cellular environment of T cells in Nrf2-null mice that results in Th1 polarization and subsequently, markedly increased production of IFNγ by activated T cells. However, the data also suggest this dysregulation is reversible over time and can be restored by wild-type antigen-presenting cells. (Supported by NIH grants DK081461, ES007079, RR016475 and ES018885).

PS 695 TBTO INHIBITS MTOR SIGNALING IN HUMAN T CELLS, A NOVEL PUTATIVE IMMUNOTOXIC MECHANISM OF TBTO.

O. L. Volger[1], J. Shao[1], A. A. Peijnenburg[1] and H. van Loveren[2]. [1]*Toxicology and Effect Monitoring, RIKILT Institute of Food Safety, Wageningen, Netherlands and* [2]*Laboratory for Health Protection Research, National Institute of Public Health and the Environment, Bilthoven, Netherlands.*

Introduction: The organotin compound tributyltin oxide (TBTO) is widely used as biocide. Compared to rodent studies where TBTO is immunotoxic by causing thymic atrophy, human populations are exposed to lower levels. The present study was set out to identify novel immunotoxic mechanisms of TBTO at a sub-cytotoxic level by focusing on TBTO-mediated alterations in the phosphorylation status of intracellular signaling proteins.
Methods and Results In the experiments TBTO was used at a sub-cytotoxic concentration of 100 nM (WST-1, ATPLite), and compared to a carrier control. Protein lysates of Jurkat cells (ATCC) that had been exposed for 0.5-24h to TBTO were hybridized on Pathscan RTK antibody arrays (CST, MA, USA). These array data showed that, after 6h and 24h, TBTO decreased the expression level of phosphorylated ribosomal protein S6 (-3-fold, N=3, P<0.05). Analyzing S6 protein expression by Western blotting and flow cytometry revealed that, after incubating for 3h, 6h, and 24h, TBTO decreased phosphorylation (-3-fold, N=4, P<0.005) without altering S6 expression. Flow cytometry showed that TBTO transiently decreased the phosphorylation of mTOR (0.5h: -1.7-fold), and P70S6K (0.5h: -2.2-fold). TBTO transiently increased phospho-AKT1/2 expression (3h: +1.3-fold). The PI3K-inhibitor Wortmannin completely abrogated the inhibition of mTOR by TBTO. TBTO elevated intracellular ROS levels (0.5-3h, N=3, P<0.05).
Conclusions: In clinic mTOR inhibitory drugs are used to elicit immunosuppression. Our study shows that a sub-cytotoxic level of TBTO inhibits mTOR signaling in human T cells in vitro. Therefore our findings indicate that TBTO exposure could lead to mTOR-mediated immunosuppression in humans. With respect to TBTO mediated mTOR inhibition, we are currently investigating if (i) ROS generation is responsible, (ii) this also occurs in human primary T cells, (iii) it has specific functional consequences for T cell-mediated immunity.

PS 696 CHARACTERIZATION OF ACUTE EFFECTS OF METHYLISOTHIOCYANATE (MITC) IN AN *IN VITRO* AND AN *IN VIVO* MODEL.

W. Tan, B. Jan, X. Deng and S. Pruett. *Department of Basic Sciences, Mississippi State University, Mississippi State, MS.*

Sodium Methyldithiocarbamate (SMD) is one of the most widely used agricultural pesticides in the U.S. Previous studies have indicated that SMD oral administration increases mortality in a mouse sepsis model and suppresses innate immunity. The major breakdown product of SMD – Methylisothiocyanate (MITC) accounts for most of the irritation symptoms caused by a major accidental spill and normal agricultural use of SMD. However, the effects of MITC have not been fully investigated. Therefore, the present study was done to characterize the acute effects of MITC on innate immune system. Considering it is the major breakdown product, the study was also to compare the effect of MITC with SMD on immunological parameters. Studies were conducted using RAW 264.7 cell model and NF-κB reporter mice model with MITC or SMD treatment, followed by challenge with LPS. A cytokine and chemokine multiplexed bead array study showed the production of many cytokines and chemokines have been suppressed after treatment with MITC or SMD at 50 μM for 2 hrs, including G-CSF, IL-13, IL-15, IP-10 MCP-1, RANTES, and others. The image analysis from NF-κB reporter mice showed that both MITC (28 mg/kg) and SMD (200 mg/kg) oral administration significantly decreased the signal strength in the treated mice, indicating NF-kB activation was inhibited. The examination on the production of cytokines and chemokines in mouse serum showed that MITC exhibited similar effect as SMD did on levels of most cytokines and chemokines, such as GM-CSF, IL-5, IP-10, and MIG. However, the changes of few cytokines and chemokines were different, such as TNFα. A mortality study indicated that MITC (28 mg/kg) caused the death faster than SMD did (50 mg/kg) during the first 48 hr-period, although the survival rate was the same after 72 hr treatment. These results indicate that MITC exhibit similar effects on immune system as SMD did. However, a further study is needed to investigate effect of MITC at lower dose, which is relevant to the exposure of human. This work is supported by NIH grant R01ES013708.

PS 697 SODIUM METHYLDITHIOCARBAMATE-INDUCED OXIDATIVE STRESS ON NF-KAPPAB REPORTER MICE.

M. Gadson, W. Tan, X. Deng and S. Pruett. *Department of Basic Sciences, Mississippi State University, Mississippi State, MS.*

As one of the most commonly used pesticides in the US, sodium methyldithiocarbamate (SMD) is reported to cause health risks in humans. This compound alters signaling and induction of cytokine production through Toll-like receptor (TLR) 4, decreasing IL-12 (Interleukin) production and increasing IL-10 production induced by lipopolysaccharide (LPS) in mice. SMD increases oxidative stress, which can contribute to the activation of NF-kB. NF-kB reporter mice were used to detect any activating form of NF-κB. Almost all pro-inflammatory cytokines require NF-kB activation. This study evaluated NF-κB activation, providing new information of reactive oxygen in the activation of NF-kB. Studies were conducted where NF-kB reporter mice will be treated with LPS at a dosage of 60 μg/mouse. Buthionine sulfoximine (BSO) decreases glutathione (GSH) synthesis and increases oxidative stress. BSO was added to drinking water at 1333 mg/l for 16 days. On the 16th day, the SMD was administered orally at a dosage of 30mg/ml and LPS by intravenous injection (60 μg/mouse) 30 minutes after SMD. After 2 hours, the mice were then imaged using the IVIS imager and samples were collected. In a similar experiment, N-acetyl cysteine (NAC, which prevents GSH depletion) was administered orally at 1g/kg for 3 days. SMD and LPS and mice were imaged as already described. Results from these experiments indicate that SMD-induced oxidative stress does not play a major role inhibition of NF-kB activation. Neither BSO + LPS nor

NAC + LPS yielded different levels of activation of NF-κB than LPS alone. However, NAC did alter production of a few cytokines, indicating biological activity. A previous study indicated either that reactive oxygen species had a complex bimodal effect on cytokine production or that oxidative stress was not the major cause of altered cytokine production. The work here demonstrates that NF-kB is not affected directly by increased reactive oxygen speciesor decreased reactive oxygen species. This work was funded by NIEHS grant R01ES013708.

PS 698 IMMUNOMODULATORY ROLES OF THE PROKARYOTIC METALLOTHIONEIN.

K. Pietrosimone[1], S. Davis[1], D. Laukens[2], D. A. Lawrence[3] and M. Lynes[1]. [1]*University of Connecticut, Storrs, CT,* [2]*University of Ghent, Ghent, Belgium and* [3]*Wadsworth Laboratory, Albany, NY.*

Metallothionein (MT) is a small cysteine rich protein. In mammalian cells, MT expression increases under stressful conditions such as exposure to divalent heavy metal cations, reactive oxygen, or reactive nitrogen. The cysteine residues in MT are essential to detoxifying reactive oxygen and nitrogen species and sequestering heavy metals. MT also shares sequence similarities with chemokines. We have shown that MT acts as a chemoattractant for human Jurkat T cells and primary leukocytes. A bacterial MT, SmtA, found in certain bacteria such as Synechococcus and Pseudomonas shares molecular features with eukaryotic MT, based on sequence comparisons and on shared antigenic epitopes that are detected by UC1MT. Unlike mammalian MT, SmtA employs both cysteine and histidine residues to bind metals. The presence of cysteine residues, the shared metal binding capacity, and the expression of shared epitopes suggests that, like MT, SmtA can detoxify reactive oxygen species and act as a chemoattractant. To test this hypothesis, a model using the pGEX-6p-SmtA plasmid transfected into Escherichia coli MC1061 has been exposed to prototype oxidants. E.coli expressing SmtA survives hydrogen peroxide exposure significantly better than E.coli containing the empty vector. These strains were used in an in vitro model of infection in which survival of the transfected E.coli was measured after co-culture with RAW264.7 macrophages. The E.coli expressing SmtA showed significantly increased survival after exposure to the RAW264.7 cells, when compared to the E.coli with empty vector cultured in the same conditions. The ability of SmtA to scavenge oxidant may provide bacteria with protection from innate immune responses that occur during the early stages of infection, and thus increase the virulence of the SmtA-expressing bacteria. Information regarding the host interaction with SmtA may also provide insight to the evolution of the relationship between MT and the immune system, and could lead to an understanding of MT's role in toxicant-mediated immunomodulaton.

PS 699 PROGRESS IN DEVELOPMENT OF PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS FOR PAHS.

S. R. Crowell[1], J. Soelberg[1], S. G. Amin[2], K. A. Anderson[3], D. E. Williams[3] and R. A. Corley[1]. [1]*Pacific Northwest National Laboratory, Richland, WA,* [2]*Penn State College of Medicine, Hershey, PA and* [3]*Oregon State University, Corvallis, OR.*

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants generated as byproducts of natural and anthropogenic combustion processes. Despite significant public health concern, physiologically based pharmacokinetic (PBPK) modeling efforts for PAHs have so far been limited to naphthalene, plus simpler PK models for pyrene, nitropyrene, and benzo[a]pyrene (B[a]P). The dearth of published models is due in part to the high lipophilicity, low volatility, and myriad metabolic pathways for PAHs, all of which present analytical and experimental challenges. Our research efforts have focused upon experimental approaches and initial development of PBPK models for the prototypic PAH, B[a]P, and the more potent, albeit less studied transplacental carcinogen, dibenzo[def,p]chrysene (DBC). For both compounds, model compartments included arterial and venous blood, flow limited lung, liver, richly perfused and poorly perfused tissues, and diffusion limited fat. Hepatic and pulmonary metabolism was described for both compounds, as were fecal and urinary clearance and protein binding. Partition coefficients for parent PAH along with their diol and tetraol metabolites were estimated using published algorithms and verified experimentally for the hydroxylated metabolites. The preliminary PBPK models were able to describe many, but not all, of the available data sets, comprising multiple routes of exposure (oral, intravenous) and nominal doses spanning several orders of magnitude. Characterization of material loss to experimental apparatus typically used in PK studies may have revealed a major source for observed inconsistencies between published data. Additional studies where material losses can be explicitly accounted for are now underway. Supported by Award Number P42 ES016465 from the National Institute of Environmental Health Sciences.

PS 700 DOSIMETRY ASSESSMENT OF SILOXANES IN COSMETIC PRODUCTS USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING.

A. Nong, R. Wang and J. Zhu. *Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada.*

Siloxanes are synthetic silicone polymers intermediates found in commercial personal care such as body lotions, antiperspirants, and shampoos. Repeated exposure of rats to high doses of decamethylcyclopentasiloxane (D5) can cause liver enlargement and induction of hepatic cytochrome P450 enzymes. Several physiologically-based pharmacokinetic (PBPK) models have been developed in the past to predict and understand the dosimetry of siloxanes through different routes of exposure. Like most siloxanes, the highly volatile nature of D5 can be emitted from products and result into the respirable air which can be a potential source of exposure. In this study, a PBPK model was developed to assess the dosimetry of D5 vapours in topically applied cosmetic products. The human PBPK model was extended to describe for all routes of exposure (skin, inhaled and oral). A personal exposure model was also included to account for emission over time of D5 off the product and skin as well as from room airflow. Concentrations of D5 in body lotion and emission levels were used to estimate the inhaled contribution of D5 vapours. The model estimated less than 3 % of the applied dose would be inhaled from body lotion emission. This work presents the application of PBPK modeling with chemical exposure data to support dosimetry estimation for the risk assessment of volatile compounds in wide range of consumer products such as siloxanes.

PS 701 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF MANGANESE EXPOSURE IN HUMANS DURING FETAL AND NEONATAL DEVELOPMENT.

M. Yoon[1], J. D. Schroeter[1], A. Nong[1], M. Taylor[2], D. C. Dorman[1, 3], M. E. Andersen[1] and H. J. Clewell[1]. [1]*The Hamner Institutes for Health Sciences, Durham, NC,* [2]*Afton Chemical, Richmond, VA and* [3]*North Carolina State University, Raleigh, NC.*

Concerns for potential vulnerability to manganese (Mn) neurotoxicity during fetal and neonatal development have been raised due to increased needs for Mn for normal growth, different sources of exposure to Mn (placental transfer, breast milk, and formula), and pharmacokinetic differences between the young and adults. A physiologically based pharmacokinetic (PBPK) model for Mn during human pregnancy and lactation was developed to predict brain Mn in the fetus and neonate. A parallelogram approach was used to build the model, using knowledge gained from the cross species extrapolation from the rat to monkey and then to human as well as the extrapolation across life stages in the rat. Key physiological processes controlling Mn kinetics during pregnancy/lactation and fetal/postnatal development were incorporated in the model based on the rat findings, which include changes in Mn uptake, excretion, and tissue specific distributions as well as placental and lactational transfer of Mn. Dynamics of human fetal and postnatal growth were incorporated. Parameters for Mn kinetics were allometrically scaled from the adult model or were estimated based on available data on Mn levels in milk, placenta, and fetal/neonatal tissues in humans. Model evaluation was performed using published cord blood and breast milk Mn and serum/erythrocyte Mn in suckling infants. Maternal Mn homeostasis during pregnancy and lactation, placenta and milk Mn, and fetal/neonatal tissue Mn for normal dietary intake and with Mn inhalation were simulated. Model predictions suggest similar or lower levels of internal exposure to Mn in the brains of fetuses/neonates compared to adults. Prediction of internal dosimetry of Mn in the target tissues of the brain is important in assessing vulnerability to this element during early life and comparing the potential impact of Mn from different sources, such as breast or cow milk, formula, food, drinking water and inhalation.

PS 702 A BBDR-HPT AXIS MODEL FOR THE LACTATING RAT AND NURSING PUP: EVALUATION OF IODIDE DEFICIENCY.

S. Li[1], M. E. Gilbert[2], R. T. Zoeller[3], K. M. Crofton[2], E. D. McLanahan[4], D. R. Mattie[5], B. C. Blount[6], L. Valentin-Blasini[6], K. Kurunthachalam[7], T. Kunisue[7] and J. W. Fisher[1]. [1]*College of Public Health, University of Georgia, Athens, GA,* [2]*NHEERL, U.S. EPA, Research Triangle Park, NC,* [3]*Biology Department, University of Massachusetts, Amherst, MA,* [4]*NCEA, U.S. EPA, Research Triangle Park, NC,* [5]*711 HPW/RHPBA, Wright Patterson Air Force Base, OH,* [6]*CCEHIP/NCEH, CDC, Atlanta, GA and* [7]*Wadsworth Center, New York State Department of Health, Albany, NY.*

The hypothalamic-pituitary-thyroid (HPT) axis controls many physiologic functions, including development and reproduction. Insufficient iodine intake is still a health concern. No adequate risk assessment tools exist to evaluate health outcomes

of chemical/dietary induced changes in serum thyroxine (T_4) concentrations in either euthyroid or sensitive populations, such as the developing fetus. Collaborative researchers are examining the effects of iodide deficiency on CNS development in the rat pup at the USEPA using propylthiouracil as a positive control. Electrophysiological, biochemical and molecular studies of the pup brain combined with behavioral studies are used for evaluating HPT mediated developmental neurotoxicity. A Biologically Based Dose Response (BBDR) HPT axis model for the lactating rat and nursing pups predicts the relationship between brain concentration of T_3 and serum concentration of T_4 as a function of iodide intake. The BBDR HPT axis model describes two negative feedback loops: 1) TSH production controlled by the brain concentration of T_3, and 2) TSH stimulation of thyroid iodide uptake and T_3 and T_4 synthesis and secretion. The model is calibrated to predict dietary iodine deficiency-induced perturbations in serum and brain thyroid hormones. The model successfully described published data sets in the dam and pup for both euthyroid and iodide deficient conditions. After successful validation for iodide deficiency, the model will be expanded to include thyroid active chemicals. The model will allow for simulation of complex dose response and exposure conditions and serve as a template for the future development of human gestation and lactation BBDR HPT axis models for use in risk assessment.

PS 703 AGE-DEPENDENT METABOLISM OF CHLOPYRIFOS AND CHLORPYRIFOS-OXON IN HUMAN PLASMA AND HEPATIC MICROSOMES.

T. S. Poet[1], C. Timchalk[1], M. J. Bartels[2] and J. N. Smith[1]. *[1]Biological Monitoring & Modeling, Battelle, Pacific Northwest Division, Richland, WA and [2]The Dow Chemical Company, Midland, MI.*

Young animals and, potentially, children may have increased sensitivity to chlorpyrifos (CPF) exposure compared to adults at higher exposure levels, which could be in part due to age differential metabolism of CPF and CPF-oxon (ultimate toxicant). In humans, there have not been studies characterizing CPF or CPF-oxon metabolism over various ages. Thus, age-dependent enzymatic metabolism of CPF and CPF-oxon were quantified in vitro using human donor hepatic microsomes (n = 30, ages 13 d-75 y) and plasma (n = 20, ages 3 d-43 y) by measuring product formation of 3,5,6-trichloro-2-pyridinol (TCPy) and CPF-oxon using gas chromatography/mass spectrometry (GC/MS). Mean hepatic CPF desulfation and dearylation Vmax values across ages were 0.35 and 0.73 nmol/min/mg microsomal protein, respectively. The mean hepatic CPF-oxon hydrolysis Vmax value was 78 nmol/min/mg microsomal protein. All hepatic measures of metabolism were consistent across ages on a per microsomal protein basis using a linear regression model ($p = 0.90$, 0.60, and 0.17, respectively). Ratios of desulfation to dearylation also did not show any age dependent relationships ($p = 0.87$). Pearson product-moment correlation coefficients of pseudo first order rate estimates and Vmax values with substrate marker activities provided by the vendor implicated CYP2B6, 3A4/5, 2C8, and 2D6 with CPF desulfation and CYP2C19 and 3A4/5 with CPF dearylation. CPF-oxon metabolism in plasma showed age-dependent increases over two factors: (1) metabolism on a per protein basis ($p = 0.03$) and (2) total plasma protein levels ($p = 0.005$). The mean overall CPF-oxon metabolism Vmax value for children < 6 mo of age was 1901 nmol/min/mL vs. 6829 nmol/min/mL for adults, > 3.5 times higher. These results are useful when evaluating potential response of different age individuals to CPF and will be integrated into a lifestage physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for CPF.

PS 704 DEVELOPMENT OF A LIFESTAGE PHYSIOLOGICALLY BASED PHARMACOKINETIC AND PHARMACODYNAMIC (PBPK/PD) MODEL FOR CHLORPYRIFOS IN RATS AND HUMANS.

J. N. Smith[1], P. M. Hinderliter[1], C. Timchalk[1], M. J. Bartels[2] and T. S. Poet[1]. *[1]Biological Monitoring & Modeling, Battelle, Pacific Northwest Division, Richland, WA and [2]The Dow Chemical Company, Midland, MI.*

Young animals and, potentially, children may have increased sensitivity to chlorpyrifos (CPF) exposure compared to adults at higher exposure levels. Thus, an age-dependent, lifestage PBPK/PD model was developed to computationally predict disposition of CPF and its metabolites chlorpyrifos-oxon (CPF-oxon) and 3,5,6-trichloro-2-pyridinol (TCPy) as well as corresponding cholinesterase (ChE) inhibition in rats and humans. In this model, age-dependent body weight was calculated from a generalized Gompertz function, and compartments (liver, brain, fat, blood, diaphragm, rapid, and slow) were scaled based on body weight from polynomial functions on a fractional body weight basis. Blood flows among compartments were calculated as a constant flow per compartment volume. Carboxylesterase activity, hepatic microsomal protein, and CPF-oxon metabolism in plasma have all demonstrated age-dependency and were scaled as such. PK of TCPy was handled as a one compartment model. Consistent with literature results, simulations of rats dosed with a single dose of 10 mg/kg CPF orally at 5, 12, and 45 days of age demonstrate increased peak CPF concentrations in blood (0.72, 0.49, and 0.40 μM) and greater maximal inhibition of plasma ChE activity (97, 93, and 77%), red blood cell ChE activity (82, 67, and 34%), and brain ChE activity (80, 56, and 25%), respectively. In humans, PBPK/PD model simulations predict similar age-dependent outcomes. For humans 6 mo, 3 y, and 30 y of age, peak CPF concentration in blood were (0.20, 0.18, and 0.17 μM), maximal inhibition of plasma ChE activity were (98, 91, and 85%), and red blood cell ChE activity were (19, 11, and 9%), respectively, after receiving a single oral dose of 0.5 mg/kg CPF. This model provides a computational framework for age comparative simulations that can be utilized to evaluate age-related responses resulting from CPF exposure.

PS 705 EFFECT OF PARAMETER VARIABILITY AND UNCERTAINTY IN A LIFESTAGE PBPK MODEL FOR CHLORPYRIFOS.

P. M. Hinderliter[1], J. N. Smith[1], M. J. Bartels[2], P. S. Price[2] and T. S. Poet[1]. *[1]Biological Monitoring & Modeling, Battelle, Pacific Northwest Division, Richland, WA and [2]The Dow Chemical Company, Midland, MI.*

Physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) models are important tools in risk assessment, allowing for the evaluation of predicted effects of chemical exposures on humans that occur as the result of real world exposures. Human populations are unique in their differences and more specifically are sexually dimorphic and exhibit a wide range in heights and weights and body composition. A lifestage model has been constructed to include growth from birth to adulthood and inter-individual variability in organ sizes and metabolic parameters. The model incorporates measured body weights (from NHANES or other published datasets) and calculates the necessary model body compartments (i.e., liver, brain, fat, blood, rapid, and slow) which are scaled based on body weight from polynomial functions on a fractional body weight basis. Variability in enzymatic parameters is included, based on measured chlorpyrifos bioactivation and detoxification in humans of different ages. To validate the model, human exposure data were simulated and matched from two controlled human oral studies. Variability was determined by calculating confidence intervals around in vitro-derived metabolic rate constants for age-dependent plasma PON1 activity. The activities measured in vitro were bootstrapped into the PBPK/PD model to account for variability and uncertainty in these parameters. As an example, RBC AChE was examined as a function of metabolic and physiological parameters using daily oral exposures from diet (CARES). The results indicate that there are no trends between RBC AChE inhibition with BMI, cardiac output, liver PON1, blood PON1, liver P450, or brain P450 in adults. Simulation of higher doses does show a trend between PON1 activities and AChE inhibition. This suggests that human variability will not substantially impact predictions of inhibition at real-world low exposures. At higher doses reduced levels of PON1 may be associated with increased inhibition.

PS 706 DEVELOPMENT OF HUMAN GESTATION AND LACTATION PBPK MODELS FOR PERFLUOROOCTANOATE (PFOA) AND PERFLUOROOCTANESULFONATE (PFOS).

A. E. Loccisano[1], M. E. Andersen[1], J. Butenhoff[2] and H. J. Clewell[1]. *[1]The Hamner Institutes for Health Sciences, Research Triangle Park, NC and [2]3M Corporation, St. Paul, MN.*

Conflicting results have been reported for associations between maternal serum/plasma concentrations of perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and human birth outcomes. Physiological changes during pregnancy such as plasma volume expansion can affect pharmacokinetics and can also be associated with birth outcomes. We have developed PBPK models for PFOA and PFOS for the gestation and lactation life stages in humans to understand more fully how the physiological changes associated with development affect tissue distributions of these compounds in the mother, fetus, and neonate. These models were derived from PBPK models for PFOA and PFOS that we previously developed for adult humans and for rats during gestation and lactation. The same model structure and parameters used in the adult human model could be used to simulate plasma concentrations in pregnant and lactating women, indicating that pharmacokinetics is not appreciably affected. Transfer of both chemicals to the human fetus was described by simple diffusion, while transfer of chemical from plasma to milk was assumed to be flow-limited. The models simulated PFOA and PFOS concentrations in maternal and fetal plasma and milk and were compared to available epidemiologic data and also used to estimate maternal exposure in the populations studied. Another application was to estimate maternal and fetal PFOA plasma concentrations in communities with contaminated drinking water. The model development identified several research needs, including the identification of transporters involved in renal resorption to explain the multi-year half-lives of these compounds in humans, factors affecting the clearance of PFOA and PFOS during gestation, and data to estimate clearance of PFOA and PFOS in the human neonate.

PS 707 LIFE-STAGE PBPK MODELS FOR MULTIPLE ROUTES OF ETHANOL EXPOSURE IN THE RAT.

S. A. Martin[1, 7], J. L. Campbell[2], K. Choi[2], H. J. Clewell[2], H. El-Masri[3, 7], W. R. LeFew[3, 7], T. E. Beasley[1, 7], W. M. Oshiro[1, 7], L. L. Degn[1, 7], P. A. Evansky[4, 7], A. Ledbetter[4, 7], J. Ford[5, 7], D. W. Herr[1, 7], W. K. Boyes[1, 7], P. J. Bushnell[1, 7] and E. D. McLanahan[6, 7]. *[1]NB/TAD/NHEERL/ORD, Research Triangle Park, NC, [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC, [3]SBB/ISTD/NHEERL/ORD, Research Triangle Park, NC, [4]ITFB/EPHD/NHEERL/ORD, Research Triangle Park, NC, [5]ACRC/RCU/NHEERL/ORD, Research Triangle Park, NC, [6]NCEA/ORD, Research Triangle Park, NC and [7]U.S. EPA, Research Triangle Park, NC.*

Ethanol is commonly blended with gasoline (10% ethanol) in the US, and higher ethanol concentrations are being considered. While the pharmacokinetics and toxicity of orally-ingested ethanol are widely reported, comparable work is limited for inhalation exposure (IE), particularly at concentrations producing blood (BEC) and brain (BrEC) ethanol concentrations associated with developmental neurotoxicity (DNT). Multi-route, life-stage PBPK models for adult, pregnant, and neonatal rats were developed and used to predict inhalation exposure concentrations yielding BEC and BrEC associated with DNT. Based on model predictions, Long-Evans rats received multiple 6-hr IE at 5,000 to 20,000ppm to assess the pharmacokinetics of ethanol. Tissues (blood, brain, eyes, liver) were collected for analysis via headspace gas chromatography to provide quantitative data and support model calibration. Kinetic time points were specific to each IE and designed to capture loading, peak, and clearance phases across low and high IE and tissue concentrations, while remaining comparable to literature data at lower concentrations. Simulations using the calibrated ethanol model were in good agreement with peak and post-exposure BEC and BrEC for most datasets, though additional work is needed to refine estimates of metabolism. This work covers a range of ethanol tissue concentrations associated with DNT in rodents, and thus may be useful for risk assessments. These models will be coupled with a gasoline model to describe biofuel blends. This abstract does not reflect EPA policy.

PS 708 ANALYSIS OF BISPHENOL A KINETICS AND METABOLISM IN NEONATAL AND ADULT MONKEYS AND RATS USING A PBPK MODEL.

J. Fisher, N. C. Twaddle, M. Vanlandingham and D. R. Doerge. *Biochemical Toxicology Division, U.S. FDA/NCTR, Jefferson, AR.*

Bisphenol A (BPA) is a widespread contaminant found in urine of over 90% of samples from NHANES. The pharmacokinetics of aglycone BPA and its primary phase II metabolite, BPA-glucuronide (BPAG) were recently reported by our laboratory in immature and mature non-human primates and rats (Doerge et al., 2010). PBPK models are under development to include this new kinetic data for aglycone BPA in the developing rat and non-human primate. First pass presystemic metabolism of orally ingested BPA (GI tract and hepatic) and enterhepatic recirculation are included in a six compartment model for BPA. BPA is metabolized by intestinal and hepatic UDPGT to BPAG. BPAG is metabolized to BPA by bacterial glucuronidase in the colon. The kinetics of BPAG are described as a two compartment sub-model. Physiological model parameters for the maturing rat were retrieved from the literature and allometric scaling was used for the monkey. These PBPK models will provide computational tools to compare the internal dosimetry of administered BPA in immature rats, which are commonly used for toxicity studies, with immature non-human primates, a species more similar to humans than rodents.

PS 709 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING (PBPK) AS A TOOL TO PREDICT 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) PHARMACOKINETICS (PK) DURING GESTATION AND LACTATION.

C. Emond[1, 2], M. J. Devito[3], M. Warner[4], B. Eskenazi[4], P. Mocarelli[5] and L. S. Birnbaum[6]. *[1]BioSimulation Consulting Inc., Newark, DE, [2]University of Montreal, Montreal, QC, Canada, [3]NIEHS & NTP, Research Triangle Park, NC, [4]School of Public Health, University of California, Berkeley, CA, [5]Department of Laboratory Medicine, University of Milano-Bicocca, School of Medicine, Hospital of Desio, Desio, Desio-Milano, Italy and [6]NCI & NIEHS, Research Triangle Park, NC.*

The PK of TCDD is relatively well understood in adult humans. TCDD induces its elimination at high exposures, while at low exposures the PK is highly influenced by the percent body fat. Our understanding of the PK of TCDD in pregnant women is less complete. To better understand the PK of TCDD in women a physiologically-based pharmacokinetic (PBPK) model was modified to include a gestational and lactational compartment and used to evaluate serum concentrations from a cohort of women of reproductive age exposed to dioxin in Seveso, Italy. The model was structured in such a way as to allow for multiple gestational scenarios observed in the general population. The gestational and lactational compartments were activated based on the reproductive profile scenario. This work compared actual serum measures of TCDD from Seveso women or from the general population with the prediction developed using the PBPK model. The initial modeling results indicate for the Seveso women, pregnancy and lactation had a small influence on the PK of TCDD, while pregnancy significantly decreased maternal blood concentrations following birth and lactation. Based on the modeling results, it appears that at high body burden, hepatic elimination is significantly induced, which masks the minor elimination from birth and milk. (The information in this abstract has been subjected to review by the NCEA, USEPA, and the NIH and the contents of the abstract does not reflect the views of these Agencies, This research was supported by funding from the U.S. Environmental Protection Agency and grants from USEPA (R82471), NIEHS (R01 ES07171), Regione Lombardia and Fondazione Lombardia Ambiente, Milan, Italy.)

PS 710 DEVELOPMENT OF PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL TO PREDICT TULATHROMYCIN DISTRIBUTION IN GOATS.

T. L. Leavens[1], L. A. Tell[2], K. A. Clothier[2], R. W. Griffith[3], R. E. Baynes[1] and J. E. Riviere[1]. *[1]Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC, [2]Department of Medicine and Epidemiology, University of California, Davis, CA and [3]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA.*

Currently, limited data and models exist to guide extrapolation of withdrawal times for approved drugs from major to minor animal species. Because PBPK models incorporate species-specific and chemical-specific parameters, they could be a useful tool to extrapolate withdrawal times of drugs across species and doses. The objective of this research was to develop a PBPK model for goats to simulate the pharmacokinetics of tulathromycin, a macrolide antibiotic. The model compartments were plasma, lung, liver, muscle, fat, kidney, and remaining poorly and richly perfused tissues. Tulathromycin was assumed to be 50% protein bound in the plasma with first order clearance to represent both fecal and urinary excretion of parent drug. Literature values were compiled for goat physiological parameters, partition coefficients were estimated from AUC ratios, and the remaining parameters were estimated by visual comparison with the experimental data. Three separate model structures were compared with plasma and tissue concentrations of tulathromycin in market age (6 mo) goats administered 2.5 mg/kg tulathromycin subcutaneously. The best simulation was achieved with a diffusion-limited PBPK model and absorption from a two compartment injection site. The model with age-appropriate physiological parameters was able to simulate plasma concentrations in juvenile (<1 mo) goats administered tulathromycin subcutaneously. The developed model and compilation of physiological parameters for goats provide initial tools that can be used as a basis for predicting withdrawal times of drugs in this minor species. [Research funded by USDA-sponsored Food Animal Residue Avoidance and Depletion Program (FARAD)]

PS 711 APPLICATION OF A MULTIROUTE HUMAN PBPK MODEL FOR BROMODICHLOROMETHANE (BDCM).

E. M. Kenyon[1], T. L. Leavens[2], C. Eklund[1] and R. A. Pegram[1]. *[1]ORD/NHEERL/ISTD, U.S. EPA, Durham, NC and [2]Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Due to its presence in water as a volatile disinfection byproduct, BDCM poses a risk for exposure via multiple routes. Mechanistic data suggest target tissue metabolism could be important for some types of BDCM-induced toxicity. Utilizing our refined PBPK model for BDCM, the impact on internal dose of BDCM from dermal and inhalation exposure during showering and bathing was compared with oral exposure from drinking water. Dose metrics evaluated were maximum blood concentration (Cmax), area under the curve (AUC) for blood BDCM, and amount metabolized in liver per hour (AML). Compartments in the model were skin, liver, gut, fat, kidney, and slowly and rapidly perfused tissue groups. Tissue transport was blood-flow limited. Metabolism occurred in the liver via two pathways (cytochrome P450, glutathione S-transferase). Chemical-specific parameters for tissue solubility and physiological parameters were obtained from the literature; metabolism parameters were estimated on the basis of our in vivo and in vitro human data. The model adequately predicted data from the published literature for oral, dermal, inhalation, and combined exposures. To produce by oral exposure (assuming a single ¼ L drink), the same internal doses that resulted from a 10-minute dermal-only exposure to water containing 10 ppb BDCM, would require the drinking water to contain 200, 470 and 7.6 ppb BDCM for Cmax, AUC, and AML, respectively. For a 10-minute, inhalation-only exposure to BDCM volatized from 10 ppb BDCM in water (estimated air concentration 2.6 ppb), the oral equivalent water concentrations were 50, 100 and 2.4 ppb for Cmax, AUC and AML, respectively. These results suggest a large contribution of inhalation and dermal exposure (e.g. from showering) to internal dose of parent BDCM reaching the systemic circulation to

be available for extrahepatic metabolism These data indicate the need to consider the contribution of multiple routes of exposure when evaluating risk. (This abstract does not necessarily reflect Agency policy).

PS 712 A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) TOOL KIT TO EVALUATE EXPOSURES TO ENVIRONMENTAL POLLUTANTS.

M. Mumtaz[1], P. Ruiz[1], M. Ray[2], J. Fisher[3] and S. Hays[4]. [1]*Department of Toxicology and Environmental Medicine, ATSDR, Atlanta, GA,* [2]*University of South Carolina, Columbia, SC,* [3]*U.S. FDA, National Center for Toxicological Research, Jefferson, AR and* [4]*Summit Toxicology Inc., Lyons, CO.*

ATSDR is mandated by Congress to identify significant human exposure levels, develop methods to determine such exposures, and design strategies to mitigate them. PBPK models have been used to evaluate toxicity of environmental pollutants through multiple exposure pathways and to study the influence of physiological state, disease state, reproductive state, life stage and life style on dosimetry and public health. ATSDR, through a cooperative agreement with the University of Georgia, has funded translational research to develop a human "PBPK model toolkit". This tool kit, when fully developed, will provide a platform that consists of a series of priority environmental contaminant models all coded in a single simulation language Berkeley Madonna. Previously, work on solvent and metal models was reported. Presented here is on-going work on models for Acrylamide and Bisphenol A. As part of this work, previously published models were slightly modified, while chemical specific parameters were retained. To evaluate model fidelity, recoded model predictions were compared with published model predictions and verified to either match simulations from previously published papers or by comparing to simulation runs in the software packages in which they were originally written. Good agreement was generally obtained for the recoded models. These models are available for ATSDR scientists and public health assessors to perform simulations of exposures from contaminated environmental media at sites of concern and to better help interpret site-specific biomonitoring data. (Supported by ATSDR Cooperative Agreement 1U01US000078).

PS 713 DERIVATION OF CHEMICAL-SPECIFIC ADJUSTMENT FACTORS FOR HUMAN VARIABILITY IN TOXICOKINETICS OF ACETONE.

G. Johanson and A. Mörk. *Work Environment Toxicology, Karolinska Institute, Stockholm, Sweden.*

BACKGROUND. In toxicological risk assessment, variability and uncertainty is dealt with by applying uncertainty or assessment factors (AF). The typical default AF for variability in the general population is 10, which may be split in 3.16 x 3.16 to account for toxicokinetic (TK) and toxicodynamic (TD) variability. Chemical-specific adjustment factors (CSAFs) may replace the default factors when quantitative TK and/or TD data are available (WHO 2005). The CSAF for TK variability is defined as the upper 90th, 95th or 97.5th percentile of the target dose in the subpopulation divided by the median target dose in the whole population. The AIM of this study was to derive CSAFs for human TK variability of acetone. METHOD. TK variability was estimated by Monte Carlo simulation, combining a population PBPK inhalation model with model parameter distributions from a Bayesian analysis of human experimental data (Mörk and Johanson, Tox Lett 2006, Mörk et al, TAAP 2009) and published distributions of age and gender related parameters. The simulations several factors: age, gender, presence/absence of endogenous acetone production and various exposure and workload conditions at work. Peak and 24-h average acetone concentrations in blood were used as target dose surrogates. Continuous exposure at 29 ppm was simulated for the general population, and 8-h daily exposure at 500 ppm for workers. RESULTS. The population-CSAFs were 1.7-2.1 excluding and 2.1-3.8 including endogenous acetone. Considerably higher CSAFs of 4.2-5.9 were obtained for children and babies. The range of worker-CSAFs was 1.3-1.9 depending on choice of percentile and exposure and workload conditions. Male and female variabilities were similar. CONCLUSIONS. The CSAFs for acetone are generally within the default AF for TK variability. A lower CSAF for workers is supported.

PS 714 PRIORITIZING TOXCAST CHEMICALS ACROSS MULTIPLE SECTORS OF TOXICITY USING TOXPI.

D. Reif, M. Martin, M. Holly, S. Gangwal, A. Wang, R. Judson, P. Volarath, R. Kavlock and D. Dix. *National Center for Computational Toxicology, U.S. EPA, Durham, NC.*

The Toxicological Prioritization Index (ToxPi™) framework was developed as a decision-support tool to aid in the rational prioritization of chemicals for integrated toxicity testing. ToxPi consolidates information from multiple domains—including ToxCast™ in vitro bioactivity profiles (a wide-ranging battery of over 500 high-throughput screening assays), inferred toxicity pathways, exposure predictions, and chemical properties/descriptors—into comprehensive toxicity scores and multivariate visualizations representing the contribution of each data domain to overall priority rankings. Here, we develop a generalizable methodology for aligning ToxCast assay data with diverse prioritization tasks and demonstrate an implementation for profiling and prioritizing chemicals according to four sectors of toxicological concern: systemic (non-cancer), cancer, developmental, and reproductive. The methodology draws upon the wealth of knowledge in existing knowledgebases to link biological pathways with relevant in vitro assay results. The multi-sector ToxPi profiles developed here can be viewed as an overall prioritization or decomposed into individual sectors for targeted evaluation. Unsupervised clustering of the ToxPi profiles segregated chemicals into sector-specific groups of toxicological concern, which revealed previously unrecognized clusters of chemicals having similar patterns of bioactivity. Exploring these chemical clusters in the context of in vivo data from ToxRefDB™ suggested testable hypotheses about the potential toxicity of chemicals without in vivo data. These results indicate that an overall weight-of-evidence approach that retains transparency as to the contribution of individual data sources can be useful in supporting prioritization decisions across diverse areas of toxicological concern. This abstract does not necessarily reflect U.S. EPA policy.

PS 715 INTEGRATION OF HIGH-THROUGHPUT SCREENING DATA WITH DOSIMETRY AND HUMAN EXPOSURE IN THE TOXICITY ASSESSMENT OF ENVIRONMENTAL CHEMICALS.

B. A. Wetmore[1], J. F. Wambaugh[2], D. M. Rotroff[2, 3], H. J. Clewell[1], M. E. Andersen[1], S. Ferguson[4], D. J. Dix[2] and R. S. Thomas[1]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC,* [2]*National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC,* [3]*Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC and* [4]*Life Technologies, Inc., Durham, NC.*

High-throughput in vitro screening and computational tools provide government an efficient way to identify those chemicals that warrant further testing while conserving limited testing resources. Incorporation of kinetic and exposure information should provide a more meaningful interpretation of in vitro findings. In this study, hepatic metabolic clearance and plasma protein binding were experimentally measured for 240 ToxCast Phase I chemicals. These data were used to parameterize a population-based in vitro-to-in-vivo extrapolation model for estimating the human oral equivalent dose necessary to produce a steady-state in vivo concentration equivalent to AC50/LEC values from ToxCast in vitro data. These values were compared against chronic aggregate human oral exposure estimates to assess whether in vitro bioactivity occurred at the anticipated maximum level of human oral exposure. Of the 170 chemicals for which exposure estimates were available, approximately 8% – including 2-phenylphenol, chlorpropham, cyromazine, dicamba, difenoconazole, piperonyl butoxide, triclopyr, and triclosan – had estimated human oral exposures greater than the predicted oral equivalent doses. If these chemicals were ranked based on AC50/LEC values alone, they would not necessarily have been high priorities for further evaluation. In vitro assay endpoints with oral equivalent doses lower than the estimated human oral exposures included changes in cell growth kinetics, cytokine expression, and cytochrome P450 isozymes. Incorporation of dosimetry and exposure information in the interpretation of in vitro screening data provides valuable information for use in determining chemical testing priorities. This abstract does not necessarily reflect EPA policy.

PS 716 A BIOLOGICALLY INFORMED FRAMEWORK FOR THE ANALYSIS OF THE PPAR SIGNALING PATHWAY USING A BAYESIAN NETWORK.

A. Beam[1, 2], W. John[1], R. Setzer[1], K. Houck[1], S. Imran[1], D. Dix[1] and R. Judson[1]. [1]*U.S. EPA/ORD/NCCT, Research Triangle Park, NC and* [2]*Statistics, North Carolina State University, Raleigh, NC.*

The US EPA's ToxCast program seeks to combine advances in high-throughput screening technology with methodologies from statistics and computer science to develop high-throughput decision support tools for assessing chemical hazard and risk. To develop new methods of analysis of these complex screening data sets, we focused on chemicals that interact with the peroxisome proliferator-activated receptor (PPAR) pathways known to mediate biological processes that are involved with chronic diseases such as diabetes, obesity, atherosclerosis, and hepatotoxicity. The ToxCast assay suite provides broad coverage of the PPAR pathways, including information on events that occur both upstream and downstream of apical chemical-PPAR interactions. We modeled the apical PPARα endpoint using a probabilistic graphical model representative of published PPAR pathways from KEGG and Ingenuity. By integrating data indicative of events occurring both upstream and

downstream of the apical endpoint of interest, we have constructed a model that represents the propagation of a signal through the PPAR pathways. Using a Bayesian analytic framework and Markov-Chain Monte Carlo (MCMC) techniques we are able to compute a posterior probability distribution for each observation in our dataset. This allowed for a probabilistic estimation of how likely a compound is to perturb a pathway, instead of relying upon a single endpoint as a sentinel for PPAR activity. This resulted in a more robust assessment of a compound's activity by lessening the impact of individual assay variance. This approach also allowed for assessment of quantities such as assay variance and measurement error, which were previously not able to be estimated in a quantitative way. Together, the model provides a general probabilistic and holistic framework for analyzing how likely a compound is to perturb a given biological pathway. This abstract does not necessarily reflect U.S. EPA policy.

PS 717 MODELING REPRODUCTIVE TOXICITY FOR CHEMICAL PRIORITIZATION INTO AN INTEGRATED TESTING STRATEGY.

M. T. Martin, T. Knudsen, K. Houck, R. Judson, R. Kavlock and D. Dix. *NCCT, U.S. EPA, Research Triangle Park, NC.*

The EPA ToxCast research program uses a high-throughput screening (HTS) approach for predicting the toxicity of large numbers of chemicals. Phase-I tested 309 well-characterized chemicals in over 500 assays of different molecular targets, cellular responses and cell-states. Of the 309 chemicals in Phase I of ToxCast, 247 have been tested in a multigeneration reproductive toxicity study and are stored in the relational database, ToxRefDB. A total of 94 chemicals were identified as reproductive toxicants, based on having achieved a reproductive lowest observed adverse effect level (rLOAEL) and having observed, at minimum, a second generation reproductive effect. Each assay was assessed for its univariate association with the identified reproductive toxicants. Significantly associated assays were used for the subsequent predictive modeling. Using linear discriminant analysis (LDA) and tenfold cross-validation, a model was produced capable of identifying reproductive toxicants with 78% accuracy (74% and 72% average training and test set balanced accuracy, respectively). Cell-free binding and cell-based transcription factor activation assays targeting steroidal and non-steroidal nuclear receptors, including estrogen, androgen, and peroxisome proliferator-activated receptors, alpha and gamma, were the major components of the model. Cytochrome P450 inhibition assays, including aromatase, and cell-based markers of growth factor stimulation were other positive indicators of reproductive toxicity potential. Assays targeting the pregnane X receptor (PXR) were the only negative indicators in the model and may provide evidence of rapid chemical metabolism and thus a lower likelihood of reproductive toxicity. The ability of this model to broadly identify reproductive toxicants, in conjunction with other predictive models spanning endocrine and developmental toxicity, will inform on chemical testing prioritization and will lead to more efficient tiered and targeted testing strategies. This abstract does not necessarily reflect U.S. EPA policy.

PS 718 BIOLOGICALLY-BASED LUMPING METHODOLOGY (BBLM) TO INVESTIGATE TOXICOLOGICAL INTERACTIONS OF COMPLEX CHEMICAL MIXTURES.

W. LeFew and H. El-Masri. *U.S. EPA, Durham, NC.*

Many cases of environmental contamination result in concurrent or sequential exposure to more than one chemical. Limitations of available resources prevent experimental toxicology from providing health risk information about all the possible mixtures to which humans or other species may be exposed. In order to increase the efficiency of risk assessment, toxicological predictions based on computational models can complement experimental efforts. We have developed a novel mathematical method, biologically-based lumping methodology (BBLM), which reduces the complexity of a mixtures model and increases computational efficiency. BBLM clusters chemicals according to similar biological properties, their metabolic rate, for instance. This method of clustering gives both a greater correlation to physical interactions and to mechanistic effects than previously utilized clustering criteria. BBLM also allows computation of error which results from lumping. By calculating the error of different lumping configurations one can find an optimal lumping configuration in the tradeoff between simplification and accuracy for a given application. To demonstrate this method, lumped equations based on a typical inhalation physiologically based pharmacokinetic (PBPK) model with metabolic competitive inhibition were developed. Two mixtures of 100 chemicals with randomly generated parameters were simulated separately for different exposure conditions. The error, computed as the average difference of simulated venous blood concentrations between the non-lumped PBPK mixtures model and the BBLM generated lumps, was examined for a range of lumping configurations. This application of BBLM produced lumping configurations of up to 90 chemicals, and the error was less than 1% of the maximum attainable venous concentration determined by the non-lumped PBPK mixtures model. This abstract does not necessarily reflect the views and policies of the EPA.

PS 719 A PBPK MODEL OF MICRO AND NANO SIZED FLUORESCENT POLYSTYRENE SPHERES IN RATS.

X. Chang[1], K. Sarlo[2], N. Walker[1], K. Blackburn[2], E. Clark[2], J. Grothaus[2] and C. Portier[1]. *[1]NIEHS, Reseach Triangle Park, NC and [2]Miami Valley Laboratories, Procter & Gamble, Cincinnati, OH.*

Knowledge on kinetics of nanomaterial is critical for assessing its potential health risks. In this study, we developed a physiologically based pharmacokinetic (PBPK) model to understand the kinetics of nanoparticles in comparison to that of chemically-identical larger particles. Four different sizes of latex fluorescent polystyrene spheres (1000, 100, 40, 20 nm) were dosed separately to rats via a single intravenous (IV) injection or oral pharyngeal aspiration (PA) into the airways. After dosing, tissue distribution was assessed over 90 days based on which a PBPK model was developed, which consists of an arterial compartment, a venous compartment, lymphatic nodes, and twelve tissue compartments, each of which described using diffusion-limited kinetics. The model takes into account the role of the lymphatic system, macrophage phagocytosis and plasma protein in sequestering particles. Using a step down procedure, we fit the data for 1000 nm spheres and used this model to predict kinetics for the other smaller spheres. The 1000 nm model adequately predicted tissue kinetics for the three nano-sized spheres in liver, lung, and heart for IV exposure. The 1000 nm model also predicted tissue kinetics well in lung, liver and gastrointestinal (GI) tract for PA exposure. However, the prediction from micro to nano failed in most of the other organs, especially in bone marrow and spleen. Through a process of elimination using statistical likelihoods, we further identified modifications to the 1000 nm model providing an adequate fit to the 100 nm data. The process was repeated at each subsequent size to identify differences in kinetics between particles. A comparison of model parameters optimized for each sized particle supports that the reticuloendothelial system plays a major role in determining the particle distribution; and the smaller particles moved more freely between blood and tissues.

PS 720 PHARMACOKINETICS-ENABLED REVERSE DOSIMETRY APPROACH IMPROVES THE ACCURACY OF BISPHENOL A (BPA) EXPOSURE ASSESSMENT.

R. Gunawan and J. Teeguarden. *Biological Monitoring/Modeling, Pacific Northwest National Laboratory, Richland, WA.*

Bisphenol A (BPA) is now a controversial monomer used in the manufacture of polycarbonate plastics and found in trace quantities in some consumer products and food containers. Unconjugated BPA is a low affinity ligand of the estrogen receptor(s) comparable to other natural estrogen receptor ligands in the food supply such as genistein and daidzein. Exposure to BPA has been widely estimated by multiplying the concentration from single urine spot samples by the estimated daily urine volume or creatinine elimination. This approach may be appropriate for chemicals that reach steady state, but for BPA, the episodic nature of dietary exposure and rapid absorption and elimination kinetics are not accounted for by the spot sampling approach. In this project, we calibrated a human physiologically-based pharmacokinetic model (PBPK) to new BPA blood and urine pharmacokinetic data following dietary exposure obtained in a clinical setting. The resulting model was exercised to estimate the oral exposure associated with spot samples from a previously conducted human oral exposure study. In contrast to the conventional spot sample method, which has errors of approximately ten folds, the PBPK-based reverse dosimetry approach can be used to calculate oral exposure to BPA with an accuracy of greater than 95%. Thus, combined with adequate data on meal times and urine sampling times, this new approach for estimating human exposure to BPA offers a significant advancement over conventional approaches.

PS 721 A SHOW OF HANDS FOR MODERN RISK ASSESSMENT: SPECIES-TO-SPECIES EXTRAPOLATIONS OF STEREOISOMERIC MIXTURES WITH PROVISIONAL PBPK MODELS.

R. Goldsmith[1], J. Kenneke[2], C. Mazur[2], D. Chang[1], Y. Tan[1], R. Tornero-Velez[1], C. Dary[3] and E. Ulrich[1]. *[1]U.S. EPA, Durham, NC, [2]U.S. EPA, Athens, GA and [3]U.S. EPA, Las Vegas, NV.*

It has been estimated that nearly 1/3 of the ~1600 agrochemicals in commerce are stereoisomeric mixtures. Modern risk assessment of these chemicals is problematic. Stereoselective PK/PD characterization and molecular asymmetry of chemicals are

not adequately addressed in the current repertoire of knowledge required for demonstrating inter-species extrapolation. In this study we have developed and evaluated de novo rat and human provisional physiologically-based pharmacokinetic (p-PBPK) models that incorporate metabolism explicitly for the stereoisomers of three conazoles; bromuconazole, triadimefon and triadimenol. The model is parameterized with an extensive metabolic in vitro dataset for the A) individual, B) enantiomerically-related, and C) diastereomerically-related stereoisomers. An extensive in silico characterization with stereoisomer resolution (3D-QSAR and 150 target molecular docking) was also performed and our in vitro / silico work-up is discussed and interpreted with relation to tissue-specific protein expression profiles and homology criteria. With these p-PBPK models we systematically explore five vital aspects of modern risk assessment: (1) "how do we deal with stereoselectivity?" (2) "how do we construct a "mixture parameter" from the isomers or interpret/incorporate "mixture parameters" from legacy data?", (3) "what is the molecular/allometric basis for animal-human extrapolations and is it "intelligent" or "convenient"?" (4) "can in silico guide in vitro efforts and can in vitro improve in silico models for stereoselective processes for PK/PD model development of chiral mixtures?" and (5) "Can stereochemistry, "a basis for sophisticated nonsense in pharmacokinetics" be incorporated into modeling to provide high-value information that reduces uncertainty in support of regulatory decision making processes and move towards a more rational science?".This work was reviewed by EPA and approved for publication but may not reflect official EPA policy.

PS 722 MARKOV CHAIN MONTE CARLO ANALYSIS OF RAT TISSUE:AIR PARTITION COEFFICIENTS.

T. Peyret and K. Krishnan. *Département de Santé Environnementale et Santé au Travail, Université de Montréal, Montréal, QC, Canada.*

Tissue-composition based mechanistic algorithms use point estimates of input parameters to predict Pta required for PBPK modeling. Characterization of distributions of input parameters, particularly, the tissue composition data has not been attempted. This study aimed to use previously published in vitro rat Pta of VOCs in Markov chain Monte Carlo simulations to analyse the variability of muscle, liver and fat composition parameters (i.e., neutral lipids NL; neutral phospholipids, PL; and water, W). A hierarchical modeling approach was used to describe the variability and uncertainty associated with tissue composition parameters. Published in vitro values of oil:air and water:air partition coefficients were used for all simulations. A two step analysis was performed: (i) using published Pta for 46 halogenated hydrocarbons, alkanes and aromatic compounds along with prior information on tissue composition (normal; mean values obtained from the literature; 20-50% variability and uncertainty); (ii) using the posterior distributions obtained following the first analysis with published data of Pta for 18 alcohol, ketones acetates and diethyl ether. For all tissues, the posterior distributions were physiologically consistent, with the mean values comparable to priors but the variability decreased (1-19 %) except for muscle and liver for which NL and PL increased (30-44 %). All analyses gave roughly the similar posterior distributions for fat and muscle content. For liver, however, the two simulations led to different posteriors for NL and PL. Overall, the results revealed that experimental data contained enough information to characterize the variability associated with rat tissue composition. The posteriors resulting from the above MCMC analysis may be used with the physicochemical characteristics of other VOCs to compute the distributions of their Pta for developing probabilistic PBPK and cellular dosimetry models (Supported by NSERC & AFSSET).

PS 723 INTEGRATED KNOWLEDGE-DRIVEN *IN SILICO* WORKFLOW IN SUPPORT OF PBPK/PD MODEL DEVELOPMENT: ESTABLISHING PRIORS FOR PYRETHROID CLASS INSECTICIDES.

X. Zhang[1], D. Chang[2], R. Goldsmith[2], J. B. Knaak[3], C. Tan[2], R. Tornero-Velez[2], J. Johnson[4] and C. C. Dary[4]. *[1]General Dynamics Information Technology, Henderson, NV, [2]NERL, U.S. EPA, Research Triangle Park, NC, [3]Department of Pharmacology and Toxicology, State University of New Yort at Buffalo, Buffalo, NY and [4]NERL, U.S. EPA, Las Vegas, NV.*

Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK/PD) Models are increasingly being used in pesticide risk assessment. However, the challenge remains in extrapolating to representative human PBPK/PD models from models constructed from collections of animal meta-data. This situation is particularly true of the pyrethroid insecticides where certain metabolism and neurotoxicity parameters cannot be measured directly in humans but must be estimated. Thus far, it has been common practice to optimize these parameter values by simulating the human tissue dosimetry data from animal data. We offer an alternative or parallel approach by estimating physicochemical and biochemical parameter values using the Physiologically Relevant Parameter Estimation (PReParE). PReParE is a web-accessible knowledge tool-kit developed within our group to facilitate both chemical and physiological parameterization of PBPK models. To evaluate how well PReParE estimated values improved PBPK model construction, we have built two PBPK models of cypermethrin, for the rat and human. The rodent model was built from parameter values optimized from animal meta-data. The human model was constructed using parameter values estimated from PReParE. The two models were compared and the differences were tested using Physiological and Anatomical Visual Analytics (PAVA). The comparison indicated that the QSAR based PReParE could enhance the rat-to-human extrapolation by augmenting initial estimates for those parameters which lack empirical data and,in turn, these estimates can serve as priors for further Bayesian analysis. Although this work was reviewed by the U.S. EPA and approved for publication, it may not necessarily reflect official Agency policy and does not represent the official views of GDIT. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

PS 724 GROUPING OF CHEMICALS IN THE 21ST CENTURY: A BIOLOGY BASED APPROACH USING METABOLOMICS.

B. van Ravenzwaay[2], M. Herold[1], H. Kamp[2], M. Kapp[2], E. Fabian[2], R. Looser[1], G. Krennrich[2], W. Mellert[2], A. Prokoudine[1], V. Strauss[2], T. Walk[1] and J. Wiemer[1]. *[1]Metanomics GmbH, Berlin, Germany and [2]Experimental Toxicology, BASF SE, Ludwigshafen, Germany.* Sponsor: R. David.

BASF has developed a very large metabolomics data base (MetaMap®Tox) containing approximately 500 data rich chemicals. This metabolome-database has been built based upon 28 day studies in rats with blood sampling and metabolic profiling after 7, 14 and 28 days after test substance treatment. Numerous metabolome patterns have been established for different toxicological targets (liver, kidney, thyroid, testes, blood, nervous system and endocrine system). With these patterns early detection of modes of action, responsible for toxicological effects can now be obtained from routine or early phase studies. Early recognition of toxicological mode of action will help to develop new compounds with a more favourable toxicological profile and will also help to reduce the number of animal studies necessary to do so. Thus this technology contributes to animal welfare by means of reduction through refinement (2R). With respect to the REACH legislation for which a large number of animal studies will need to be performed, one of the most promising methods to reduce the number of animal experiments is grouping of chemicals and read-across to those which are data rich. So far mostly chemical similarity or QSAR models are driving the selection process of chemical grouping. However, 'omics technologies such as metabolomics may help to optimize the chemical grouping process by providing biologically based criteria for toxicological equivalence. This is not only based on the recognition of modes of action (specific metabolome patterns) but also by comparing the entire metabolome finger print of a chemical with those of the 500 reference compounds. This provides a ranking of chemicals based on their metabolome profile. The combined evaluation of specific mode of action patterns with chemical ranking based on the entire finger print is a powerfull tool for biology based grouping of chemicals. Going from QSAR to QBAR (quantitative biological activity relationship).

PS 725 STATISTICAL APPROACHES TO ANALYZING A LARGE, MULTI-TREATMENT, TIME COURSE STUDY OF METAL PELLETS IMPLANTED INTO MOUSE MUSCLE—CLINICAL MEASUREMENTS AND PREDICTIVE MODELING.

W. Bao[2], C. Tsu-Ming[2], R. D. Wolfinger[2], E. J. Perkins[3] and D. I. Bannon[1]. *[1]Directorate of Toxicology, U.S. Army Public Health Command, APG, Aberdeen, MD, [2]SAS Institute, Inc., Cary, NC and [3]Environmental Laboratory, U.S. Army Engineer Research and Development Center, Vicksburg, MS.*

In a time course study of the effects of a single implanted pellet (Nickel, Tungsten/Nickel/Cobalt Alloy, Tantalum, or Control) in mice; over 400 Illumina Sentrix MouseRef-8 v1.1 Expression Bead Chip Arrays were generated to assess the effect of treatments on gene expression profiles over time. We present solutions for several data analysis challenges associated with such a large dataset. First, transformation: there are a significant number of genes with negative intensity values. We investigate shifted log and missing value imputation approaches. Second, normalization: there is an obvious batch effect associated with the 5 labeling plates. We explored different normalization methods, including batch correction. Third, differentially expressed genes: We use a mixed model ANOVA approach to account for correlations over time, to estimate time course profiles, and to test their differences. Finally, predictive modeling: We used cross validation model comparison with eight different predictive models to perform further analysis.

PS 726 UPDATED ALUMINUM PHARMACOKINETICS IN INFANTS FOLLOWING EXPOSURES THROUGH DIET AND VACCINATION.

R. J. Mitkus, M. O. Walderhaug and M. Hess. *U.S. FDA/CBER, Rockville, MD.*

Aluminum is a ubiquitous element that is released into the environment via volcanic activity and the natural breakdown of rocks on the earth's surface. Exposure of the general population to aluminum occurs primarily through the consumption of food, antacids, and buffered analgesics. Exposure to aluminum in the general population can also occur through vaccination, since vaccines often contain aluminum salts (frequently aluminum hydroxide or aluminum phosphate) as adjuvants. Since some lay public have hypothesized that aluminum in vaccines may pose a risk to infants, we wanted to develop an up-to-date analysis of the safety of aluminum adjuvants. We therefore conducted a literature search and found that in an effort to evaluate the relative contribution of childhood vaccines and diet to aluminum levels in infants, Keith et al. (2002) previously analyzed the pharmacokinetics of aluminum for infant dietary and vaccine exposures and compared the resulting body burdens to the minimal risk levels (MRLs) established by the Agency for Toxic Substances and Disease Registry (ATSDR). We updated the analysis of Keith et al. (2002) with a current pediatric vaccination schedule recommended by the US Advisory Committee on Immunization Practices, a more recent aluminum retention function from human volunteers, and an adjustment for the kinetics of aluminum efflux at the site of injection. Using these updated pharmacokinetic parameters we found that the body burden of aluminum from vaccines and diet throughout an infant's first year of life is one to three orders of magnitude lower than the "safe level" calculated using the MRL for dietary aluminum. We conclude that episodic exposures from vaccines that contain aluminum adjuvant have been and continue to be extremely low risk to the pediatric population and that this contributes to the high benefit of continued aluminum adjuvant use in vaccines.

PS 727 ASSESSING THE ROBUSTNESS OF CHEMICAL PRIORITIZATIONS BASED ON TOXCAST CHEMICAL PROFILING.

A. Wilson, S. Gangwal, M. Martin, R. Judson, D. Dix and D. Reif. *National Center for Computational Toxicology, Office of Research and Development, U.S. EPA, Research Triangle Park, NC .*

A central goal of the U.S. EPA's ToxCast™ program is to provide empirical, scientific evidence to aid in prioritizing the toxicity testing of thousands of chemicals. The agency has developed a prioritization approach, the Toxicological Prioritization Index (ToxPi™), that calculates a comprehensive toxicity potential and a relative priority rank by incorporating information from ToxCast in vitro bioactivity data (high-throughput screening results from over 500 diverse assays), inferred toxicity pathways, in vitro to in vivo dosimetry estimates, chemical structural descriptors, and exposure considerations. Here, we explore the robustness of the prioritization assessing potential endocrine activity of 309 chemicals in the face of several sources of variation: 1) changes in the chemical makeup of the experiment, 2) missing data, and 3) spurious (false-positive) assay results. Bootstrap resampling was used to assess the effects of alternative chemical sets. Although missing data was not an issue in Phase I, it may be a concern in subsequent phases and in certain data domains (e.g. exposure data). To address this concern, we simulated both missing-at-random and missing-by-domain datasets for comparison with complete data. A similar approach was taken to assess the potential impact of false-positive assay results. Generally, the higher-scoring chemicals tended to be less sensitive to alternative chemical sets but were more sensitive to missing values and false positives than lower-scoring chemicals. However, initial results for all experiments showed 95% confidence intervals with mean width representing less than one decile, indicating that the multivariate endocrine rankings are relatively stable in the face of anticipated levels of common sources of data variation. This robustness, which is essential to a reliable prioritization scheme, arises out of the comprehensive nature of the scores, in that no single datum wields ultimate influence. *This abstract does not necessarily reflect U.S. EPA policy.*

PS 728 TOXPLORER™: A COMPREHENSIVE KNOWLEDGEBASE OF TOXICITY PATHWAYS USING ONTOLOGY-DRIVEN INFORMATION EXTRACTION.

I. Shah[1], A. Singh[2], C. Haugh[1], J. Jack[1], R. Judson[1], T. Knudsen[1], M. Martin[1] and J. Wambaugh[1]. *[1]NCCT, U.S. EPA, Research Triangle Park, NC and [2]Lockheed Martin, Research Triangle Park, NC .*

Realizing the potential of pathway-based toxicity testing requires a fresh look at how we describe phenomena leading to adverse effects *in vivo*, how we assess them *in vitro* and how we extrapolate them *in silico* across chemicals, doses and species. We developed the ToxPlorer™ framework to extract experimental evidence from the literature about the effects of chemicals in living systems and to coherently synthesize prior knowledge into semantic networks. This was accomplished in four main steps. First, we developed an ontology to formally describe functional relationships in toxicology, which include molecules and their interactions, but also cell types, cellular processes and behaviors, phenotypes and histological effects. Second, we systematically analyzed the text of 655,271 PubMed abstracts about the mammalian liver using 363,472 diverse entities in our ontology. Third, we used this information to focus on a subset of 23,244 abstracts about nuclear receptor-mediated hepatocarcinogenesis. Out of 241,944 sentences a subset of 3,712 sentences from 2,199 abstracts produced more than 100,000 putative relationships of relevance. Fourth, we used ontology-driven information extraction tools to find less than 5,000 semantically-valid assertions about chemical-induced hepatotoxicity. By manually curating this information we found evidence relating 501 chemicals, 671 genes, 121 cell-events and 38 histological lesions (to date). Our findings recapitulate many of the events involved in nuclear receptor-mediated direct and indirect hyperplasia, formation of preneoplastic foci and development of neoplastic lesions in rodents. ToxPlorer™ is publicly available along with tools for analyzing and interactively reconstructing putative toxicity pathways.
This abstract does not necessarily reflect US EPA policy.

PS 729 BELIEF THEORY IN MECHANISTIC PHARMACOVIGILANCE: USING THE BIOMEDICAL LITERATURE TO CONFIRM ADVERSE DRUG EVENT REPORTS FROM ELECTRONIC HEALTH RECORDS.

E. Ahlberg Helgee and S. Boyer. *Computational Toxicology, AstraZeneca and EU-ADR Consortium, Mölndal, Sweden.*

Mining of electronic health records (EHRs) for indications of adverse drug events carries with it a high false positive rate. In order to assess possible biological mechanisms underlying these positive 'signals' the EU-ADR project has embarked on an extensive program of biomedical literature mining to enhance the interpretation of adverse event signals from the 35 million European EHRs used in the project. We have assessed the utility of belief theory to objectively combine the signals arising from EHRs and from the biomedical literature into a single 'confirmed signal'. Diverse sources of evidence such as this can be combined into a single predictor in a number of ways, such as simple consensus voting methods, weighted consensus estimates and full probability Bayesian estimates. However, few methods have the ability to handle both full probability 'objects', like Bayesian probability models, and models in which the underlying probability structure is partly or completely unknown. The combination of EHR-based signals and the biomedical literature, however, contains a mixture of full probability estimates and partial/unknown probabilities. Combining these types of evidence therefore requires a mathematical structure that manages both types of probability expressions. Dempster-Shafer theory deals with measures of "belief" as opposed to probability. It also introduces an explicit formulation for the unknown or uncertain state. We have applied Dempster-Shafer Belief Theory to a number of known signals from EHR data including upper GI bleeding and rhabdomyolysis. The results suggest that this approach can be used to combine signals from EHRs with biomedical text mining in order to build a robust, biological mechanism-based pharmacovigilance system that could enhance the ability to simultaneously monitor both EHRs and the literature to provide earlier warning of adverse events following drug treatment.

PS 730 PREDICTING ADAPTIVE RESPONSE TO FADROZOLE EXPOSURE: COMPUTATIONAL MODEL OF THE FATHEAD MINNOWS HYPOTHALAMIC-PITUITARY-GONADAL AXIS.

M. Breen[1, 2], D. Villeneuve[1], G. Ankley[1], K. Watanabe[3], M. Breen[1], A. Lloyd[2] and R. Conolly[1]. *[1]U.S. EPA, Research Triangle Park, NC, [2]North Carolina State University, Raleigh, NC and [3]Oregon Health and Science University, Beaverton, OR.*

Exposure to endocrine disrupting chemicals can affect reproduction and development in both humans and wildlife. We are developing a mechanistic mathematical model of the hypothalamic-pituitary-gonadal (HPG) axis in female fathead minnows to predict dose-response and time-course (DRTC) behaviors for endocrine effects of fadrozole. The model includes two feedback regulatory loops within the HPG axis that mediate adaptive responses to endocrine stress. One regulatory loop controls the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion from brain, and the other regulates LH and FSH receptor recycling in ovary. Fathead minnows were exposed to fadrozole at 3 or 30 ug/L for 8 days followed by a 20-day recovery phase, with samples collected for plasma 17β-estradiol (E2) and vitellogenin concentrations during exposure and post-exposure. Adaptive changes in plasma E2 levels occurred during exposure and overshoot occurred post-exposure. Comparing the model-predicted DRTC with experimental

data provided insight into how the feedback control mechanisms embedded in the HPG axis mediate these changes. As this work progresses we will obtain a refined understanding of how adaptive responses within the HPG axis of fathead minnows affect DRTC behaviors for fadrozole. This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect Agency policy.

PS 731 APPLICATION OF A HUMAN PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL TO EVALUATE BENZENE BLOOD LEVELS FOR PUBLIC HEALTH CONCERN.

D. A. Fowler[1], R. R. Worley[1], J. S. Wheeler[1], C. Welsh[1], D. Moffett[1] and J. Fisher[2]. *[1]Agency for Toxic Substances and Disease Registry, CDC/ATSDR/NCEH, Atlanta, GA and [2]National Center for Toxicological Research, U.S. FDA, Jefferson, AR.*

ATSDR recently evaluated exposure to benzene and related petroleum VOCs in two neighborhoods located near petroleum refineries and a major interstate. Each resident provided a blood sample that was analyzed for benzene levels and smoking biomarkers. We used a human physiologically-based pharmacokinetic (PBPK) model for benzene (multi-route, multi-compartment, developed in Berkeley Madonna simulation software) to model possible exposure scenarios resulting in measured benzene blood levels and evaluate the public health significance of those benzene blood levels. Measured benzene blood concentrations were an order of magnitude higher in smokers relative to non-smokers. Because benzene is rapidly metabolized, benzene blood concentrations predominantly reflect recent exposures preceding blood collection. We modeled peak and steady state inhalation of benzene resulting in the observed benzene blood levels. By doing so, we were able to evaluate PBPK predicted inhalation exposures that could result in the measured benzene blood concentrations with respect to health guidelines. Our comparative analyses included ATSDR's chronic inhalation minimal risk level (MRL) and the Benchmark Concentration lower bound estimate (BMCL). Measured benzene blood levels in non-smokers correspond to modeled exposures that were below ATSDR's chronic inhalation MRL and do not suggest a public health hazard. Blood from smokers had higher levels of benzene that correlated with smoking biomarkers, suggesting an elevated benzene blood level association with smoking. These elevated benzene levels correspond to modeled exposures above the MRL and near the BMCL, suggesting a health concern for smokers. This model application demonstrates the combined use of a human PBPK model and biomonitoring data in enhancing exposure analysis and public health decision-making.

PS 732 CHARACTERIZATION OF STRAIN- & DOSE-DEPENDENT ROUTES OF ELIMINATION FOR BENZENE & ITS METABOLITES IN 4 MOUSE STRAINS.

G. A. Knudsen[1], R. K. Kuester[1], M. L. Cunningham[2], J. E. French[2] and I. G. Sipes[1]. *[1]Medical Pharmacology, University of Arizona, Tucson, AZ and [2]National Toxicology Program, National Institutes of Environmental Health Sciences, Research Triangle Park, NC.*

Benzene & its metabolites are eliminated in the urine, feces or expired-air, in a dose-dependent manner. To characterize the strain- & dose-dependent routes of elimination for benzene, four strains were selected based on data from previously reported PK studies in 18 isogenic strains: C57BL/6J (reference), NZW/LacJ (high blood, marrow AUC), PWK/PhJ (highest marrow AUC) & FVB/NJ (lowest blood, marrow AUC). In C57BL/6J, elimination patterns were similar for doses 0.1-10 mg/kg (3-5% expired, 4-7% feces, 70-88% urine); at 100 mg/kg, 30% was expired while 66% micturated. At 1,000 mg/kg, 70% of the dose was expired with the remainder recovered in urine. In all doses tested (0.1-100 mg/kg), elimination in NZW/LacJ, PWK/PhJ, & FVB/NJ mice was similar to C57BL/6J mice, with few exceptions. All strains of mice eliminated the majority of administered [14C] radioactivity in the urine (NZW/LacJ: 80-88%; FVB/NJ: 75-85%; PWK/PhJ: 70-77%). Fecal recovery for NZW/LacJ was similar to C57BL/6J (3-7%), while FVB/NJ mice eliminated more on average (7-11%). Doses recovered in feces were highest in PWK/PhJ (9-13%); however, at the highest dose, recovery was 4%. In C57BL/6J, NZW/LacJ, & FVB/NJ, expired [14C] benzene did not change significantly over the low doses (3-7%). In PWK/PhJ, a 10 mg/kg dose resulted in significantly more expiration of the dose when compared to the same dose in FVB/NJ & NZW/LacJ ($p<0.05$). All strains expired significantly more benzene (18-30%) at 100 mg/kg ($p<0.05$; no inter-strain differences were detected due to lack of statistical power). HPLC-radiometric analyses of expired air samples detected a single peak that co-eluted with [14C]-benzene. These data indicate small but statistically significant strain- & dose-dependent differences in benzene elimination at low doses. This research was supported in part by the NIEHS NTP Grant No. N01-ES-45529 & Southwest Environmental Science Center Grant No. P3-ES-06694.

PS 733 VIRTUAL LIVER: EVALUATING THE IMPACT OF HEPATIC MICRODOSIMETRY FOR TOXCAST CHEMICALS.

J. F. Wambaugh[1], J. Jack[1], C. Haugh[1], J. Zaldívar Comenges[2] and I. Shah[1]. *[1]National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC and [2]Institute for Health and Consumer Protection, European Commission, Joint Research Centre, Ispra, Italy.*

The U.S. EPA's ToxCast™ program uses hundreds of high-throughput, *in vitro* assays to screen chemicals for potential toxicity. The assays are used to probe *in vitro* concentrations at which target cellular pathways and processes are perturbed by these chemicals. The U.S. EPA's Virtual Liver (v-Liver™) is a cellular systems model of hepatic tissues providing simulated *in vivo* context for *in vitro* data. The modeling framework used assumes that each cell is capable of independently responding to its local microenvironment – if that environment varies then cells are expected to behave differently. A spatially-extended hepatic lobule model is used in conjunction with a physiologically-based pharmacokinetic (PBPK) model in order to link whole-body exposure to cell-scale concentrations. Depending upon the rate of metabolism, the concentration throughout a given lobule can be either homogenous (similar to what might be found in a high-throughput assay well) or hetereogeneous, in which case hepatic behavior may be altered depending on spatial location, *i.e.* hepatic zone. For a subset of the ToxCast chemicals, both the rate of human metabolism by hepatocytes and the fraction of chemical bound to protein in plasma have been measured *in vitro*, allowing the rate of metabolic clearance to be estimated. By incorporating these estimated values into the simulation model for zonal variability, we determined that exposure to bisphenol A, myclobutanil, simazine, diethylhexyl phthalate (DEHP), and caffeine all may result in heterogeneous cell-level exposures within the hepatic lobule. For these chemicals, a spatially-extended approach such as v-Liver, may be needed to reconcile *in vitro* results with *in vivo* consequences. *This abstract does not necessarily reflect U.S. EPA policy.*

PS 734 MOLECULAR DYNAMICS STABILITY STUDY OF THE WILD-TYPE AND MUTANTS OF THE HEPTAPEPTIDE (NNFGAIL) SEGMENT OF AMYLIN AGGREGATE.

W. M. Berhanu[1, 2] and A. E. Masunov[1, 2]. *[1]Chemistry, University of Central Florida, Orlando, FL and [2]Nanoscience Technology Center, University of Central Florida, Orlando, FL.*

In this study, we performed in silico mutation analysis to examine the stability of the heptapeptide NNFGAIL segment from amyline peptide. The model system is one of the shortest fragments that can form amyloid fibrils similar to those formed by the full length peptide. The mutants obtained by single glycine replacement were also studied to investigate dry self-complementary interface between the neighboring β-sheet layers. The results of molecular dynamics simulations for 10ns, shows that some mutant models were found to be unstable, and their stabilities were largely dependent on the position of replaced residues. Our mutational simulation is in agreement with the pervious experimental observations. Furthermore, we estimated the free energies in solution. The analysis of the binding free energy reveals the determining role of van der Waals and hydrophobic forces within the dehydrated interface, which can serve as quantitative measure of shape complementarities between the side chains. Total binding free energy for the wild type is more favorable, than for the mutants. The present study provides the atomic level understanding of the aggregation behavior and the driving force for the amyloid aggregates of the NNFGAIL segment of amylin. The result of our simulation indicated computation mutation in combination with MD simulation is useful in identifying the critical amino acid that stabilizing the NNFGAIL amyloid peptide and could be useful for rational design of amyloid inhibitors and amyloid-specific biomarkers for diagnostic purposes for Type II diabetes.

PS 735 MECHANISTIC UNDERSTANDING OF DRUG EFFECTS USING METABOLOMICS: A COMPUTATIONAL APPROACH BASED ON A RAT LIVER MODEL.

O. Shaikh, S. Das, R. Kumar, S. Raghavan and K. Subramanian. *Strand Life Sciences, Bangalore, India.*

Understanding the biochemical mechanisms by which drugs interact with the liver is an essential component in preventing DILI. Here, we describe a method based on mathematical model of rat liver metabolism to identify drug-pathway interactions using metabolomics. The predictive dynamic model consists of enzymes and transporters encompassing carbohydrate, glutathione and fat metabolism in the rat liver. To determine how different processes affect overall metabolism, we performed

global parameter sensitivity analysis (GSA) on various model parameters and studied their impact on several key metabolites such as ATP, mitochondrial membrane potential, GSH, NADH, Acetyl CoA, Malonyl-CoA, Cholesterol, DG and TG. Model parameters are varied by 10%, and the changes in metabolite profiles are quantified. This analysis allows parameters to be ranked in order of their impact on liver metabolism.

We mimicked drug-induced perturbations on the rat liver by changing the kinetic parameters of sensitive enzymatic reactions either singly or in combination and obtained metabolite profiles. An optimization problem was formulated to estimate the values of parameters that were potentially responsible for this metabolite profile. An objective function was defined to minimize the error between predicted and observed metabolite profiles of key metabolites described above. The estimated values of the parameters were then compared with the values used in the simulations and found to match well. This provides a mechanistic basis for relating metabolite changes to specific pathways being deranged by a drug. This approach works well for both steady state and time-varying metabolite profiles.

PS 736 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR RDX (HEXAHYDRO-1, 3, 5-TRINITRO-1, 3, 5-TRIAZINE) IN FEMALE B6C3F1 MICE.

M. L. Gargas[1], C. P. Gut[1], M. R. Okolica[1], A. Ntamack[1], L. M. Sweeney[1], L. R. Williams[2], G. Reddy[2] and M. S. Johnson[2]. *[1]Naval Medical Research Unit - Dayton, Wright-Patterson AFB, OH and [2]U.S. Army Public Health Command, Aberdeen Proving Ground, MD.*

RDX is an explosive used in military applications and has been detected in ground water surrounding US military installations and at manufacturing facilities. The current cancer slope factor was estimated from the incidence of liver neoplasms in female mice (no cancers were found in either sex of rats chronically exposed to RDX) and is based on outdated animal to human scaling techniques and mouse tumor data that has subsequently been revised using more current pathological criteria. In addition, dose-response was not evaluated using Benchmark Dose (BMD) approaches. There is a need to derive an updated cancer potency factor for RDX using more current data and techniques, including the use of internal dose and BMD modeling. The purpose of the work reported here was to finalize the development of a PBPK model for RDX kinetics in the female mouse. An initial PBPK model structure for female mice was constructed using an existing model for rats and existing data on tissue partition coefficients. The rate of RDX metabolism for female mice was determined experimentally in our laboratory using hepatic microsomal preparations and the resulting rate constant was incorporated as a PBPK model parameter. Model verification was performed by comparing model predictions to blood and liver concentration time-course data collected in our laboratory from female mice orally dosed with RDX at target doses of 1.5, 7, or 35 mg/kg. The cancer potency of RDX in humans will be determined in future evaluations using the female mouse PBPK model developed in this current work together with a PBPK model for RDX kinetics in humans.

PS 737 BIOMARKERS OF EXPOSURE *VERSUS* PBPK MODEL ESTIMATES OF INTERNAL DOSE: A CASE STUDY WITH ACRYLAMIDE.

R. S. DeWoskin. *U.S. EPA, Research Triangle Park, NC .*

Estimates of internal dose greatly improve the confidence in risk assessments for systemic toxicants when extrapolating dose-response relationships across species, routes of exposure, subpopulations, or study conditions (e.g., in vitro to in vivo). Two leading methods to estimate internal dose are: 1) biomarker of exposure models, and 2) physiologically based pharmacokinetic (PBPK) models. Hemoglobin (Hb) adducts are good biomarkers of exposure to acrylamide (AA) and its mutagenic metabolite, glycidamide (GA). The U.S. EPA posted an IRIS assessment for acrylamide that estimated internal blood levels in rats and humans with an AA-Hb and GA-Hb adduct model. Concurrent with the posting of the IRIS assessment, an updated AA PBPK model was published providing an opportunity to compare results, and to evaluate the advantages each method has for specific applications. The Hb-adduct model and the PBPK model gave similar results for the ratio of the rat external dose (mg/kg/day) to the equivalent human external dose of AA (i.e., the dose that would produce comparable internal levels of AA and GA in blood). External dose ratios were in the 4.5-6.5 range for comparable levels of AA in blood, and in the 0.75-1.0 range for GA in blood. The ratio for AA in blood is similar to the value from a body weight scaling (BW 2/3 or 3/4), while the lower ratio for comparable GA internal levels reflects the considerably different production and clearance of GA in humans compared with rats (i.e., BW scaling would grossly overestimate this ratio). The Hb-adduct model only estimates internal dose of AA or GA in blood. Thus, when blood levels suffice as a surrogate dose metric for an adverse effect, the simpler seven parameter Hb-adduct model is preferred (i.e., based on AIC) to the nine compartment, 68 parameter PBPK model. The PBPK model, however, estimates a variety of dose metrics (e.g., unbound AA or GA in blood and tissues), and provides more functionality for extrapolating risks and hypothesis testing. [The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA]

PS 738 *IN VITRO* 3, 5-DICHLOROANILINE NEPHROTOXICITY IN FRESHLY ISOLATED RAT RENAL CORTICAL CELLS.

C. Smurthwaite, S. Baksi, T. Ferguson, C. Schuetz, D. Anestis and G. O. Rankin. *Pharmacology, Physiology, and Toxicology, Marshall University, Huntington, WV.*

Chloroanilines are widely used in manufacturing dyes, drugs, agricultural chemicals and industrial intermediates. Previously, we demonstrated that 3,5-dichloroaniline (3,5-DCA) induced nephrotoxicity in vivo in rats and in vitro in a rat renal cortical slice model. In this study, freshly isolated renal cortical cells (IRCC) obtained from male Fischer 344 rats were used to investigate the nephrotoxic potential of 3,5-DCA and to examine if metabolites and/or oxidative stress contributed to 3,5-DCA-induced nephrotoxicity in vitro. Nephrotoxicity was determined by incubating IRCC (~4 million cells/ml; 3 ml total volume) with vehicle (dimethyl sulfoxide, DMSO; 30 microL) or 3,5-DCA (0 - 1.5 mM) for 60, 90, or 120 minutes and calculating lactate dehyrogenase (LDH) release as a percent of total LDH. In some experiments, IRCC were pretreated with antioxidants (2.0 mM ascorbate, 1.0 mM glutathione, 1.0 mM alpha-tocopherol, or 2.0 mM N-acetyl-L-cysteine) to explore the role of oxidative stress or pretreated with a cyclooxygenase inhibitor (1.0 mM indomethacin), CYP inhibitor (1.0 mM metyrapone or 1.0 mM piperonyl butoxide) or FMO inhibitor (1.0 mM methimazole or 2.0 mM n-octylamine) to explore the role of metabolites in 3,5-DCA nephrotoxicity. 3,5-DCA nephrotoxicity was evident at 120 min at 0.5 mM 3,5-DCA and by 60 min with higher 3,5-DCA concentrations. All pretreatments reduced 3,5-DCA toxicity to varying degrees. These results suggest that 3,5-DCA is nephrotoxic to IRCC in a time and concentration dependent manner, metabolites contribute to 3,5-DCA nephrotoxicity, and oxidative stress may be a mechanism for inducing 3,5-DCA nephrotoxicity. This work was supported by NIH grant 3P20RR016477-09S1.

PS 739 *IN VITRO* ANALYSIS OF CYCLOSPORINE A-INDUCED NEPHROTOXICITY BY GENE EXPRESSION AND ^{1}H-NMR-METABONOMICS OF CELL LYSATES.

K. Herrgen[1], O. Schmal[1], C. J. Burek[1], M. Gruene[2], P. Jennings[3] and W. Dekant[1]. *[1]Department of Toxicology, University of Wuerzburg, Wuerzburg, Germany, [2]Department of Organic Chemistry, University of Wuerzburg, Wuerzburg, Germany and [3]Department of Physiology and Medical Physics, Medical University of Innsbruck, Innsbruck, Austria.*

Early detection of nephrotoxic side effects of drug candidates combined with reduction of animal testings is still a challenge for preclinical drug development. Since some renal biomarker candidates, like KIM-1 and LCN-2 (NGAL) performed well in vivo, the scope of this study was to test their performance in vitro in human proximal tubular epithelial cell lines. To gain additional information, the intracellular metabolite patterns were tracked by ^{1}H-NMR-Metabonomics and compared to the gene expression data. In the present study, Cyclosporine A (CsA) was used as a model compound for drug induced nephrotoxicity. Differentiated RPTEC/TERT1 and A498 cells were used as cell models and drug treatment was performed up to 6 days by repeated dosing.

A multivariate data analysis of the hydrophilic cell lysates examined by ^{1}H-NMR revealed a clear discrimination between cytotoxic and non-cytotoxic dose groups. The corresponding metabolites are linked to energy consumption as well as alterations of renal osmolytes. This can be interpreted as cellular dedifferentiation, a known effect of Cyclosporine A. Regarding the analyzed biomarker genes, only LCN-2 and GADD153 showed an upregulation. Other markers, like KIM-1 or Clusterin, did not show consistent alterations in this experimental set up. The increased expression of the mechanistic marker GADD153 confirms decompensated ER stress as an early event in proximal tubular cell damage caused by CsA.

Effective CsA concentrations were monitored by LC-MS and revealed a bound part of about 20% of the nominal concentration along with varying intracellular concentrations. This highlights the importance of concentration measurements for in vitro testings.

PS 740 EVALUATION OF MRP2 TRANSPORTER-MEDIATED PROTECTION AGAINST CISPLATIN-INDUCED NEPHROTOXICITY USING *IN VITRO* AND *IN VIVO* APPROACHES.

X. Wen[1], M. Goedken[2], C. D. Klaassen[3], J. E. Manautou[4] and L. M. Aleksunes[1]. [1]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ,* [2]*Pathology, Merck & Co., Inc., Lafayette, NJ,* [3]*Pharmacology, University of Kansas Medical Center, Kansas City, KS and* [4]*Pharmaceutical Sciences, University of Connecticut, Storrs, CT.*

Use of the chemotherapeutic drug cisplatin is limited in part by nephrotoxicity. Prior work has demonstrated that the organic cation transporter 2 (OCT2) is responsible for uptake of cisplatin into renal tubule cells. We hypothesized that the multidrug resistance-associated protein 2 (MRP2) effluxes cisplatin metabolites into urine thereby limiting intracellular accumulation and subsequent nephrotoxicity. In the present study, cisplatin disposition and toxicity were assessed in MRP2- and OCT2-transfected HEK293 cells (human embryonic kidney cells) and also in male wild-type and Mrp2-null mice. Cisplatin induced cytotoxicity in HEK293 cells at 72 h with an IC50 value of about 4 μM. More potent toxicity was observed in OCT2-expressing cells (IC50= 1 μM), whereas less toxicity (IC50=7 μM) was found in MRP2-expressing cells. To study the in vivo role of MRP2 in the renal efflux of cisplatin, serum and kidneys were collected from wild-type and Mrp2-null mice between 10 min and 4 days after cisplatin treatment (15-23 mg/kg ip). At 3 and 4 days, plasma BUN levels were 2.4-fold higher in Mrp2-null mice than wild-type mice. Histopathological analysis confirmed proximal tubule degeneration and necrosis in cisplatin-treated mice, with more extensive injury observed in Mrp2-null mice. Increased susceptibility of Mrp2-null mice to cisplatin toxicity correlated with higher renal platinum levels (27% and 47% at 24 and 48 h, respectively) compared to wild-types. Notably, Oct2 protein expression was similar between wild-type and Mrp2-null mice. Collectively, these data suggest that OCT2 and MRP2 are responsible for the renal secretion of cisplatin and deficiency in Mrp2 increases platinum accumulation and susceptibility to cisplatin-induced kidney injury in mice (Supported by DK-080774).

PS 741 MODULATION OF CISPLATIN MEDIATED OXIDATIVE STRESS BIOMARKERS BY RESVERATROL.

M. Valentovic, J. G. Ball and J. Brown. *Pharmacology, Physiology, and Toxicology, Marshall University School of Medicine, Huntington, WV.*

Cisplatin is associated with a serious dose limiting nephrotoxicity as a major limiting side effect. The development of interventions to retard or prevent cisplatin associated renal toxicity would be of immediate human relevance. Resveratrol (RES) is a naturally occurring phytochemical which displays both anticancer and antioxidant properties. This study examined whether alterations in oxidative stress markers were an early event of cisplatin toxicity that occurred prior to the loss of membrane integrity. Male Fischer 344 rats (200-250 g) were anesthetized with isoflurane and the kidneys were isolated, decapsulated and rinsed in 3 ml ice cold Krebs buffer. Renal cortical slices were prepared and 50-100 mg of tissue was pre-incubated with 30 ul ethanol (VEH) or 30 ug/ml resveratrol (RES, final concentration) for 30 min at 37oC under an oxygen atmosphere. The tissue was incubated for 120 min in 3 ml oxygenated Krebs containing 0, 75, or 150 ug/mL cisplatin in an oxygen atmosphere under the same conditions. Loss of membrane integrity was evaluated as leakage of lactate dehydrogenase (LDH). Cisplatin associated oxidative stress was evaluated by measuring total, Cu/Zn and Mn Superoxide dismutase (SOD), glutathione peroxidase and catalase enzyme activity in renal cortical slices. The protective effect of RES was evaluated following a 30 min pre-exposure. LDH leakage required a 120 min exposure to cisplatin. GSH peroxidase activity was diminished by cisplatin when compared to vehicle control beginning at 60 min. Total SOD activity was also diminished by cisplatin beginning at 60 min. A 30 min pre-incubation with RES reduced oxidative stress by maintaining GSH peroxidase enzyme activity. Total SOD enzyme activity was preserved by RES co-incubation with cisplatin. These findings indicate that RES pre-incubation diminishes cisplatin renal toxicity and early changes in oxidative stress prior to the onset of LDH leakage. (Supported by NIH Grant INBRE 3P20RR016477-09S4).

PS 742 URINARY BIOMARKERS FOR THE EARLY PREDICTION OF CISPLATIN-INDUCED CHANGES IN KIDNEY FUNCTION AND INJURY IN RATS.

Y. Chen, D. Brott, P. Bentley, D. Thurman, L. Kinter and R. Bialecki. *Safety Assessment, AstraZeneca Pharmaceuticals, Wilmington, DE.*

Cisplatin (CDDP) is a common chemotherapy treatment for solid tumors; however, nephrotoxicity remains a major adverse effect of its clinical use. Current diagnosis of drug-induced acute kidney injury (DIKI) relies on detection of elevated serum creatinine (SCr) and blood urea nitrogen (BUN) levels, delayed biomarkers of acute insult. Our aim was to evaluate if CDDP-induced changes in multiple DIKI biomarkers preceded decrements in renal hemodynamics (GFR, RPF) and histopathology changes in the same animal using a novel integrated pharmacology platform. The platform consists of an automated blood sampling and radio telemetry (ABST) system with continuous infusion and quantitative urine collection capability to measure renal hemodynamics and nephron site-specific DIKI biomarkers (KIM-1, RPA-1, albumin, α-GST, GSTYb1, lipocalin, clusterin and osteopontin) in surgically prepared conscious male Wistar rats (n=8). After a single administration of CDDP (15 mg/kg, i.p.), renal hemodynamics, excretory function, and DIKI biomarkers were measured on days 1, 2 and 3 via 24-hour urine collection samples. Renal tissue was examined microscopically at study termination. Cisplatin induced significant increases in SCr and BUN (5.9- and 7.2-fold vs. baseline, respectively; $P < 0.05$), profound decreases of GFR and RPF (-50.2% and -25.3% vs. baseline, respectively; $P < 0.05$), and severe renal tubular damage at day 3. DIKI biomarkers increased progressively throughout the study (maximum fold increases vs. baseline: α-GST, 103; GSTYb1, 260; clusterin, 36; albumin, 208; KIM-1, 15; $P < 0.05$ ea.). All animals showed increased DIKI biomarkers that preceded decreased GFR and RPF, or structural damage. These findings suggest that early increases in DIKI biomarker excretion may predict DIKI risk following acute exposure to nephrotoxicants. The integrated pharmacology platform provides a unique approach to detect the risk of DIKI during early drug discovery.

PS 743 STATE-OF-THE-ART DETECTION OF NEPHROTOXICITY BY URINARY PROTEIN BIOMARKERS.

B. Emde, P. Hewitt, S. Czasch, F. v. Landenberg and T. C. Fuchs. *Toxicology, Merck Serono, Darmstadt, Germany.*

In the pharmaceutical and chemical industry the kidney is routinely assessed during preclinical safety evaluations. The role of the kidney as a central detoxification organ leads to an extraordinary exposition of renal tissue to drugs, reactive metabolites or environmental compounds. This exposition leads to cell damage, primarily due to high blood flow, xenobiotic metabolism and clearance. It has been reported that the frequency of drug-induced renal toxicity, relative to other toxicities, is 2 – 20%. The reason for this variation may be due to the difficulty of assessing renal toxicity by traditional markers, which are insensitive, as is the case with blood urea nitrogen and serum creatinine.
For improved detection of nephrotoxicity, a panel of novel urinary kidney biomarkers was recently approved (2008) by the US-FDA and EMA. One newly developed assay is based on the Luminex® xMAP® multiplexing technology. To establish this Widescreen™ rat kidney toxicity assay for routine testing, rats were treated with Cisplatin, Vancomycin or Puromycin up to 28 days. The candidate proteins, Kim-1, Timp-1, VEGF, Osteopontin, Clusterin, Cystatin C, Calbindin, β2 microglobulin, GST-α and NGAL, were measured in urine. Additionally, the RENAstrip® dipstick assay for Kim-1 was tested. For interpretation of the data standard clinical pathology and histopathological investigations were performed. The results showed a time and/or dose dependent increase in several biomarkers. A specific time pattern was also identified for these urinary markers. Histopathologically, all compounds showed dose-related kidney effects. Cisplatin and Vancomycin targeted the tubules, whereas Puromycin primarily showed glomerula damage. The urinary biomarker data correlated well to the expected areas of damage within the Nephron caused by these model toxicants. The sensitivity and specificity, calculated by the ROC curves, indicate a high diagnostic potential of these new in-life biomarkers. A comparison of Kim-1 detection by two different technologies confirmed the benefit for its use in routine pre-clinical studies.

PS 744 URINARY FIBRINOGEN: A MECHANISTIC TRANSLATIONAL BIOMARKER FOR VASCULAR DYSFUNCTION AND INFLAMMATION IN THE KIDNEY.

D. Hoffmann[1], A. Krishnamoorthy[1], V. Ramirez Gonzalez[1], Q. Zhang[2], P. L. Goering[2], R. B. Brown[2], S. Waikar[1] and V. S. Vaidya[1]. [1]*Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA and* [2]*CDRH, U.S. FDA, Silver Spring, MD.*

Fibrinogen (Fg) mRNA and protein is significantly up regulated in the kidney following acute kidney injury (AKI) in mice, rats and humans. We hypothesized that urinary fibrinogen (uFg) may serve as a biomarker for kidney vascular dysfunction and inflammation. In a rat model of renal tubular and vascular damage induced by 30 min bilateral renal ischemia, we observed ~80-fold increase in uFg concentration after 24h (peak at 72h of ~300-fold) following reperfusion, correlating with histopathologic injury and elevated urinary kidney injury molecule-1 (Kim-1) and N-acetyl glucosaminidase (NAG). Similarly, in a mouse model of nephrotoxicity

and renal inflammation induced by 20 mg/kg cisplatin, ip, significantly increased levels of uFg (~4-fold) and Kim-1 (~15-fold) were detected as early as 24 h that peaked at 72 h (~20-fold for both). In contrast, a rat model of gentamicin (50, 100, 200, or 300 mg/kg, sc, for 3 days) induced proximal tubular damage in the absence of vascular dysfunction and inflammation, resulted in significant increases in urinary Kim-1 and NAG, but did not result in any change in kidney Fg mRNA or urinary Fg. Furthermore, in a human cross-sectional study, a ~2000-fold increase in uFg was observed in 10 patients with clinically established multifactorial AKI versus healthy volunteers (n=10) compared to a 20-fold excretion of urinary Kim-1 and NAG. In a longitudinal follow-up of 2 patients, who underwent surgical repair of abdominal aortic aneurysm and developed AKI after surgery, increased levels of uFg were detected earlier than changes in serum creatinine, which supports the potential use of uFg as predictive biomarker of renal vascular injury. In conclusion, we report uFg as a sensitive and specific translational biomarker for tubulovascular inflammation in the kidney providing mechanistic information about the cause of AKI.

PS 745 METABOLOMICS ANALYSIS SUGGESTS ROLE OF 3-INDOXYL SULFATE IN CENTRAL NERVOUS SYSTEM TOXICITY AND AN EARLY INDICATOR OF CHEMICALLY-INDUCED RENAL FAILURE.

M. V. Milburn[1], D. Alexander[1], J. R. Zgoda-Pols[2], S. Chowdhury[2], M. Wirth[2] and K. B. Alton[2]. *[1]Metabolon, Durham, NC and [2]Department of Drug Metabolism and Pharmacokinetics, Merck Research Laboratories, Kenilworth, NJ.* Sponsor: G. Vansant.

An investigative renal toxicity study was conducted with Compound A using LC-MS and GC-MS metabolomics to identify small biomarkers of acute renal failure (ARF) that could aid in a better mechanistic understanding induced ARF in mice. The metabolomics study revealed 3-Indoxyl sulfate3 (3IS) as the most sensitive marker of renal toxicity. 3IS is a known renal toxin that accumulates in blood of uremic animals or patients due to a decreased or absent urinary excretion of 3IS during renal failure. The accumulation of uremic toxins such as 3IS in blood and tissues can also cause multiple physiological changes involving central nervous system (CNS) dysfunction, such as uremic encephalopathy. Compound B, a close structural analogue of Compound A, was used as a negative control since it did not cause ARF in mice and cisplatin (CDDP) was used as a positive control. An LC-MS-based bioanalytical assay for the determination of 3IS in mouse matrices was also developed. Following treatment with nephrotoxicants (Compound A or CDDP), 3IS levels were markedly increased in murine plasma and brain, thereby contributing to renal- and CNS-related toxicities. Furthermore, as expected urinary excretion of 3IS appeared to be absent in those animals due to compromised renal function. Thus, these data suggest that 3IS could potentially serve as a marker of renal and CNS toxicities during drug-induced ARF in mice. Furthermore, this comprehensive approach that includes untargeted metabolomic and targeted bioanalytical sample analyses could be used to investigate toxicity of other compounds that pose preclinical or clinical development challenges in a pharmaceutical setting.

PS 746 SHORT-TERM TOXICITY OF MELAMINE AND ITS MIXTURE WITH CYANURIC ACID IN F344 NEONATAL RATS AND SEARCHING FOR NOVEL KIDNEY BIOMARKERS.

H. Kang[1], Y. Park[1], S. Jeong[2], J. Seo[1], Y. Jean[1], J. Kang[1], H. Shin[1] and S. Son[1]. *[1]Veterinary Toxicology and Chemistry, National Veterinary Research and Quarantine Service, Anyang, Republic of Korea and [2]GLP Research Center, Hoseo University, Asan, Republic of Korea.*

This study was performed to compare the systemic toxicity between Mel alone and Me+Cya mixture treated to neonatal F344 rats. 1-day-old male and female F344 rats were administered with Mel (0, 10, 35, and 118 mg/kg bw), Cya (150 mg/kg bw), and Mel+Cya (0.35+0.35, 1.0+1.0, and 3.5+3.5 mg/kg bw) via gavage once per day for 4 weeks. Clinical signs, pathological findings, clinical chemistry and urine test were examined after the treatment. Kidney toxicity biomarkers (KIM-1, TIMP-1, VGEF, clusterin, NGAL, osteopontin, β2m, GST-α, calbindin and cystatin C) in serum and urine were analyzed. While there were no gloss pathological findings in all of treatment groups, relative weight organs of thyroid glands and epididymis were significantly decreased at Mel+Cya (1.0+1.0 and 3.5+3.5). Serum total protein and albumin were increased by Mel+Cya (0.35+0.35, 1.0+1.0, and 3.5+3.5). Serum uric acid was decreased by Mel (10, 35, and 118) and Cya. Round shaped and greenish dark-brown colored crystals were detected in urine sediment at Mel+Cya (3.5+3.5). Also, polygonal and translucent crystals were found in rats treated with Mel treatment (118). TIMP-1 in serum was decreased in male and female rats by Mel+Cya (3.5+3.5). Cystatin C in serum was increased in female rats by Mel+Cya (3.5+3.5). Osteopontin in serum was decreased in male rats by Mel+Cya (3.5+3.5). Urinary TIMP-1 and clusterin increased in female rats by Cya (150) and Mel+Cya (3.5+3.5), respectively. The NOAEL of Mel+Cya is estimated as 0.35 mg/kg bw/day based on the relative weight organs of thyroid gland and epididymis. The threshold level of renal toxicity in terms of urinary crystal formation in Mel+Cya could be between 1.0 mg/kg and 3.5 mg/kg bw/day while in Mel treats could be between 35 and 118 mg/kg. Conclusively, this study supports that Mel mixture could also show more toxicity especially in male neonate rats than Mel or Cya alone.

PS 747 TRANSCRIPTION LEVELS OF BIOMARKERS OF ACUTE RENAL INJURY IN F344 RATS CO-EXPOSED TO MELAMINE AND CYANURIC ACID FOR SEVEN DAYS.

L. Camacho, K. Kelly and G. Gamboa da Costa. *Division of Biochemical Toxicology, National Center for Toxicological Research/U.S. FDA, Jefferson, AR.*

The intentional adulteration of pet food ingredients with melamine and its derivatives, including cyanuric acid, was responsible for the renal failure and death of a substantial number of cats and dogs in the USA. Experimental evidence demonstrates that the co-exposure to low levels of both compounds elicits renal toxicity due to the formation of melamine cyanurate crystals in the kidney nephrons. In this work, we investigated if a co-exposure to melamine and cyanuric acid in rats leads to alterations in the gene expression of proteins (KIM1, TIMP1, clusterin, osteopontin, and NGAL) that have been proposed as urinary biomarkers for acute kidney injury. Male and female F344 rats were fed NIH41 diet supplemented with 0, 69, 229, or 694 ppm each of melamine and cyanuric acid, which resulted in an exposure of 0, 8.6, 17.6, and 29.8 mg/kg bw/day melamine and cyanuric. Histopathology and clinical chemistry examination indicated marked toxicity in the animals exposed to the two highest doses (17.6 and 29.8 mg/kg bw/day) but not at the lowest dose (8.6 mg/kg bw/day). Quantitative PCR analysis of kidney tissue indicated increased expression of genes coding for KIM1, TIMP1, clusterin, and NGAL relative to the control in animals co-exposed to 17.6 and 29.8 mg/kg bw/day melamine and cyanuric acid. These data suggest that the change in expression of these genes may constitute an endpoint to assess combined toxicity of melamine and cyanuric acid in rat kidneys. Funded in part by NTP IAG 224-07-0007 between the FDA/NCTR and the NIEHS/NTP.

PS 748 THE USE OF FLOW CYTOMETRY TO STUDY MECHANISMS OF MITOCHONDRIAL BIOGENESIS IN RENAL CELLS.

V. Kale and R. Schnellmann. *Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Mitochondria play an important role in the outcome of toxicant exposures and ischemia reperfusion injury. Mitochondrial biogenesis is tightly regulated by mitochondrial and nuclear transcriptional factors and results in increased mitochondrial mass/number. We treated renal proximal tubular cells (RPTC) with three chemicals that induce mitochondrial biogenesis, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 5-HT2 agonist); SRT1720 (SIRT1 activator); and metformin (AMPK activator). We then isolated mitochondria and determined mitochondrial membrane potential (TMRM), cardiolipin (NAO) content, and relative size (side scatter, SSC) using flow cytometry. Among the three chemicals tested, DOI (10 μM) increased NAO (16±7-%), and TMRM (19±8-%) staining, and decreased mitochondrial size and complexity (indicated by an increase in the geometric mean of SSC (289±55, 575±112)) over vehicle controls after 24 h treatment. Metformin (1 mM) and SRT1720 (10 μM) treatment of RPTC did not alter NAO staining or TMRM staining. However, SRT1720 decreased the geometric mean for SSC (280±21, 196±33) indicating increased mitochondrial size and decreased in the complexity. Mitochondrial biogenesis was confirmed in each case by demonstrating that mitochondrial complex proteins (ATPsynthase β & NDUFB8), mitochondrial copy number and RPTC respiration increased. In conclusion, flow cytometry is a useful tool to study individual mitochondria following chemical exposures and mitochondrial biogenesis. Although all chemicals caused mitochondrial biogenesis only DOI increased cardiolipin staining and membrane potential suggesting that different inducers of mitochondrial biogenesis cause different mitochondrial phenotypes. Funding: NIGMS (GM084147).

PS 749 OXIDANT INJURY INDUCES RAPID DEGRADATION OF MITOCHONDRIAL CALPAIN 10 IN RENAL PROXIMAL TUBULE CELLS.

M. A. Smith and R. G. Schnellmann. *Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC.*

Calpain 10, which is found in the cytosol and the mitochondrial matrix, has been shown to cleave proteins in the electron transport chain and ATP synthesis. Previously, we showed that knockdown of calpain 10 causes renal proximal tubule

(RPTC) cell death, indicating that calpain 10 is required for viability. The goal of these experiments was to ascertain if mitochondrial calpain 10 levels decrease following toxicant exposure and determine the mechanism of mitochondrial calpain 10 degradation. Primary cultures of RPTC were treated with 0.4 mM tert-butylhydroperoxide (TBHP), 0.1 mM cisplatin, 5 μM A23187 or diluent. In addition, isolated mitochondria or matrix were pretreated with 10 μM MG132, 10 μM epoxomicin, 10 μM calpeptin, 10 μM CYGAbuK (calpain 10 inhibitor), Protease inhibitor cocktail (1:100 according to manufacturer's instructions) or diluent and incubated for various times. Calpain 10 was measured by immunoblot analysis in mitochondria, matrix and cytosolic fractions. RPTC treated with the oxidant TBHP, resulted in a 52% decrease in mitochondrial calpain 10 protein levels at 45 min and remained decreased at 24 hr. In cisplatin-treated RPTC, mitochondrial calpain 10 levels decreased 35% only at 24 hr. A23187-treated cells showed no change in mitochondrial calpain 10 levels. In isolated mitochondria, calpain 10 protein levels decreased 60% and 70% at 30 and 120 min, respectively. Using various protease inhibitors, only MG132 blocked calpain 10 degradation in mitochondria and matrix. These results demonstrate that oxidant injury in RPTC causes a rapid degradation and prolonged reduction in mitochondrial calpain 10 levels. In contrast, cisplatin and A23187 did not elicit a robust oxidant injury and mitochondrial calpain 10 levels did not decrease extensively. The protease inhibitors provide evidence that calpain 10 is degraded in the matrix by Lon protease. The loss of mitochondrial calpain 10 following oxidant injury is consistent with reports that Lon protease degrades oxidized proteins in the matrix. Supported by NIH ES012239.

PS 750 MITOCHONDRIAL HOMEOSTASIS AFTER ACUTE KIDNEY INJURY: SUSTAINED ALTERATIONS IN MITOCHONDRIAL PROTEINS, FUSION/FISSION, AND BIOGENESIS.

J. A. Funk, B. E. Shaner and R. G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Mitochondrial dysfunction is a pathological process that occurs after acute kidney injury (AKI), and at this time the state of mitochondrial homeostasis during the initiation and recovery phases of AKI remains unclear. Thus, we examined changes in mitochondrial respiratory proteins, fusion/fission processes, and biogenesis in a non-lethal, rat model of myoglobinuric AKI. AKI was induced by glycerol injection into the hind limbs of male SD rats. Rats had reduced kidney function as determined by a rise in SCr levels from 0.5 to 2 mg/dL 24h after injection. Recovery of kidney function initiated between 24 and 72h with SCr levels decreasing to 1.3 mg/dL at 72h, and continued to decrease to control levels at 144h. Cleaved caspase-3 was observed in glycerol-treated rats between 24 and 144h post-injury. Respiratory proteins NDUFB8, ATP synthase β (ATPβ), and cytochrome c oxidase subunit I (COXI) were decreased from 24 to 144h after injection, suggesting loss of mitochondria and function. Mitochondrial-encoded genes ND6 and COXI mRNA were decreased at 72h and 144h, whereas mRNA levels for the nuclear-encoded genes NDUFB8 and ATPβ either did not change (NDUFB8) or were elevated throughout the study (ATPβ). Expression of mitochondrial fission/fusion proteins, dynamin-related protein (Drp1) and mitofusin 2 (Mfn2), were elevated at 24h and throughout the course of the study. We observed increases in markers of mitochondrial biogenesis, including elevations in PGC-1α, PGC-1 related coactivator (PRC), and nuclear respiratory factor (NRF)-1 mRNA levels and elevations in PGC-1α, NRF-1, and (Tfam) protein levels at 72h and 144h. These findings reveal that following injury mitochondrial biogenesis and dynamic processes undergo sustained activation. However, there is a persistent loss of mitochondrial proteins and function after AKI that is associated with differential regulation of transcription within the nucleus and the mitochondria. Funding by NIH GM084147.

PS 751 IDENTIFICATION AND CHARACTERIZATION OF MITOCHONDRIAL TOXICANTS.

L. P. Wills, G. C. Beeson, R. Trager, C. Lindsey, Y. K. Peterson, C. C. Beeson and R. G. Schnellmann. *Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

Many environmental, pharmaceutical, and industrial compounds negatively affect human health through deleterious effects on mitochondrial function. Mitochondrial toxicity is defined as a decrease in the function and /or number of mitochondria, leading to decreased respiration and energy production. Loss of mitochondrial function can result in organ damage due to cellular injury, apoptosis, or necrosis. Currently there is no database of chemicals that cause mitochondrial dysfunction and there are no reliable methods for predicting mitochondrial toxicity. We hypothesized that discrete toxicophores defined by distinct chemical entities can identify previously unknown and future mitochondrial toxicants. To examine this hypothesis we conducted a novel respirometric screen using primary cultures of rabbit renal proximal tubule cells (RPTC) and the Seahorse Biosciences XF analyzer. Primary cultures of RPTC are ideal for this assay because they exhibit mitochondrial function similar to that found *in vivo*. A total of 1760 compounds, 1280 chemicals from the Sigma Library of Pharmacologically Active Compounds (LOPAC) and a random 480 compound subset from the ChemBridge DIVERSet of 50,000 small molecules, were tested in the screen at a concentration of 5 μM. The screen identified 22 compounds that decrease uncoupled respiration, a stress test for mitochondrial function. The effects of the potential mitochondrial toxicants (0.5-5 μM) on cell viability were examined. Of the 22 compounds tested, six compounds decreased uncoupled respiration prior to cell death. One cluster of toxicants was aligned to identify a preliminary toxicophore of mitochondrial dysfunction. We postulate that this approach will enable the identification of mitochondrial toxicants and the prediction of mitochondrial toxicity for both drug discovery and risk assessment. Supported by T32 CA119945-04 and GM084147.

PS 752 MITOCHONDRIAL GSH STATUS IN COMPENSATORY RENAL HYPERTROPHY DUE TO UNINEPHRECTOMY AND DIABETIC NEPHROPATHY.

L. H. Lash, B. Benipal, D. A. Putt and Q. Zhong. *Pharmacology, Wayne State University School of Medicine, Detroit, MI.*

Renal proximal tubular (PT) cells exhibit compensatory cellular hypertrophy after either reductions in functional renal mass or in diabetic nephropathy. We have used both the uninephrectomized (NPX) rat and streptozotocin (STZ)-treated diabetic rat to study the biochemistry and toxicology of compensatory renal hypertrophy. PT cells in both models exhibit increases in rates of mitochondrial respiration and activities of several enzymes ('hypermetabolic' state), which is associated with enhanced oxidative stress and results in greater susceptibility to many forms of chemically induced injury. In both models, concentrations of GSH are also elevated in both cytoplasm and mitochondria, suggesting a compensatory response. The mitochondrial GSH pool is determined by function of 2 inner membrane transporters (the dicarboxylate [DIC] and 2-oxoglutarate [OGC] carriers). These are electroneutral exchangers that transport dicarboxylates in exchange for inorganic phosphate or 2-oxoglutarate in exchange for other dicarboxylates, respectively. To determine the source of the elevated levels of mitochondrial GSH, mRNA and protein expression as well as transport activities were characterized. In both models, transport function was clearly increased for both carriers although no differences were found in mRNA expression. In remnant kidney from NPX rats, no differences were found in protein expression of either carrier. In renal mitochondria from diabetic rats, however, while no differences were observed in expression of the OGC, DIC protein levels were substantially higher (~ 2-fold) in samples from diabetic vs. control rats. Hence, although levels of GSH in kidneys are elevated in renal mitochondria from both NPX and diabetic rats, mechanisms underlying the increases differ. In NPX rats, the major mechanism appears to be an increase in mitochondrial dicarboxylate content due to the hypermetabolic state. In diabetes, in contrast, there is also an increase in DIC protein levels, not due to a change in transcription but due to increased protein stability or decreased turnover.

PS 753 THE CYTOPROTECTIVE EFFECT OF N-ACETYLCYSTEINE AGAINST ROS-INDUCED CYTOTOXICITY IS INDEPENDENT OF ITS ABILITY TO ENHANCE GLUTATHIONE SYNTHESIS.

F. Zhang, S. S. Lau and T. J. Monks. *Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.*

2,3,5-tris(Glutathion-S-yl)-hydroquinone (TGHQ), a metabolite of hydroquinone (HQ), is toxic to renal proximal tubule epithelium cells. TGHQ retains the ability to redox cycle and create an oxidative stress. Consequently, we determined whether the antioxidant, N-acetylcysteine (NAC) could protect HK-2 cells from TGHQ-induced toxicity to assist in elucidating the contribution of reactive oxygen species (ROS) in TGHQ-induced toxicity. NAC provided remarkable protection against TGHQ-induced toxicity to human kidney proximal tubule epithelial cells (HK-2). NAC almost completely inhibited TGHQ-induced cell death, mitochondrial membrane potential collapse as well as ROS production. NAC also attenuated TGHQ-induced DNA damage, and the subsequent activation of PARP and ATP depletion. Moreover, NAC significantly attenuated JNK and p38-MAPK phosphorylation induced by TGHQ. Interestingly, NAC alone markedly increased ERK1/2 activation, and the upstream MEK inhibitor, PD-98059, only partially attenuated this activation, suggesting that NAC can directly activate ERK1/2 activity. However, although NAC is frequently utilized as a GSH precursor, the cytoprotection afforded by NAC in HK-2 cells was not a consequence of increased GSH levels, since inhibition of GSH synthesis with BSO had no effect on NAC-mediated cytoprotection. We speculate that NAC might exert its protective effect in part by directly scavenging ROS and in part via ERK1/2 activation (funding sources: P30ES006694).

PS 754 PLASMA, URINE, AND TISSUE CONCENTRATIONS OF HEXAVALENT CHROMIUM ASSOCIATED ACUTE KIDNEY INJURY IN SPRAGUE-DAWLEY RATS.

R. P. Brown[1], Q. Zhang[1], D. S. Barber[2], A. C. Komiyama[1] and P. L. Goering[1]. *[1]CDRH, U.S. FDA, Silver Spring, MD and [2]University of Florida, Gainesville, FL.*

Hexavalent chromium (Cr) is a well-known nephrotoxic agent in both experimental animals and humans. The dose of Cr that produces acute kidney injury (AKI) in experimental animals is well understood; however, dose information is rarely available for humans exposed to Cr, especially in patients with an orthopedic implant made from a Cr-containing alloy. Instead, the concentration of Cr in serum, plasma and/or urine is typically reported in these individuals. Since the histopathology of Cr-induced AKI is similar in rodents and humans, it would be useful to characterize the concentration of Cr in plasma, urine and kidney tissue in rodents associated with the onset on AKI as well as the administered dose that produces these effects. In this study, potassium dichromate was administered to male, Sprague-Dawley rats by a single SC injection in doses of 2.5, 5.0 and 10 mg Cr/kg. Rats were placed in metabolism cages and urine was collected for 18 hrs. Serum biomarkers (BUN and blood creatinine) and urinary renal injury biomarkers (α-GST, GSTYb1, RPA-1, clusterin, KIM-1, NGAL, osteopontin and NAG) were measured. Cr concentrations in plasma, urine and kidney were assessed using ICP-MS. A statistically significant increase in levels of some urinary biomarkers (α-GST, GSTYb1, clusterin, Kim-1, NGAL) was observed following administration of 10 mg/kg which corresponded to increases in blood creatinine (NOAEL = 5 mg/kg). No change was seen in levels of BUN, osteopontin, or RPA-1 at any dose level. The mean urinary Cr concentration (n=4) was 0.01, 17.64 and 38.56 μg/ml in control, NOAEL and LOAEL group rats, respectively. Levels of Cr in tissue and plasma also exhibited a dose-dependent increase. Since data on the dose of Cr associated with AKI are available in rats but not humans, and data on the concentration of Cr in various matrices are readily available in humans but not rats, these data provide a means to compare the interspecies effects of Cr on the kidney.

PS 755 COVALENT MODIFICATIONS OF KIDNEY PROTEINS BY S-(1, 2-DICHLOROVINYL)-L-CYSTEINE SULFOXIDE (DCVCS): *IN VITRO* CHARACTERIZATION USING N-BIOTINYL-DCVCS (NB-DCVCS).

R. M. Irving[1], M. S. Brownfield[2] and A. A. Elfarra[1, 2]. *[1]Molecular and Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI and [2]Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI.*

DCVCS is a reactive electrophile and potent nephrotoxicant generated by the oxidative metabolism of the trichloroethylene metabolite S-(1,2-dichlorovinyl)-L-cysteine (DCVC). DCVCS can covalently modify macromolecules, a property that likely plays a role in its potent nephrotoxicity. In this study, biotin-tagged DCVCS (NB-DCVCS) was synthesized and its reactivity toward glutathione (GSH) and kidney proteins was characterized. When NB-DCVCS was incubated at physiological conditions (pH 7.4, 37°C) with GSH (3:10 molar ratio), four covalent adducts were detected by HPLC and characterized by ESI-MS as monoadducts resulting from Michael addition-elimination reactions (addition of GSH followed by elimination of HCl) between NB-DCVCS and GSH. The half-lives of the two NB-DCVCS diastereomers in the presence of GSH were 19.2 and 42.3 minutes, whereas the half-lives for the DCVCS and N-acetyl DCVCS diastereomers were 19.4 and 3.8 minutes, and 9.9 and 23.1 minutes respectively. When rat kidney cytosol was incubated with 312.5 nM to 10 μM NB-DCVCS for 3 hours at 37°C and immunoblotting was used to detect the biotin tag, a dose-dependent increase in signal with different molecular weights was observed, demonstrating that NB-DCVCS covalently binds to multiple kidney proteins. These results provide the first compelling evidence that DCVCS can form adducts with kidney proteins. Furthermore, these studies indicate that this novel reactive electrophilic probe, derived from a known nephrotoxicant, shows promise as a useful reagent for future studies on characterization of the covalent modification of proteins by nephrotoxic cysteine S-conjugates. (Supported by NIH RO1 DK 044295 and NIEHS T32-ES-007015)

PS 756 ZINC DEFICIENCY EXACERBATES DIABETES-INDUCED RENAL OXIDATIVE DAMAGE, INFLAMMATION, AND FIBROSIS.

B. Li[1, 2], L. Miao[2] and L. Cai[1]. *[1]University of Louisville, Louisville, KY and [2]Nephrology, Jilin University, Changchun, jilin, China.*

Zinc (Zn) as an essential trace element has numerous physiological functions, particularly as an antioxidant to protect the cells and organs against various oxidative damages. However, Zn deficiency often occurs in the patients with diabetes; what is the effect of Zn deficiency on diabetic kidney has not been addressed. The purpose of the present study was to examine the effects of Zn deficiency on diabetes-induced renal oxidative damage, inflammation and fibrosis in streptozotocin (STZ)-induced type 1 diabetic mice. Type 1 diabetes was induced in FVB mice treated with multiple low doses of STZ at 50 mg/kg body weight daily for five days. Five days after the last injection of STZ, STZ-treated mice with hyperglycemia and age-matched normal mice were treated with and without Zn chelator, TPEN at 5 mg/kg daily for four months. Renal oxidative damage, inflammation and fibrosis in these mice were examined by histopathological observation, Naphthol AS-D Chloroacetate esterase assay, immunofluorescent staining, and Western blotting assay. Results: Chronic treatment with TPEN for four months significantly decreased systemic Zn levels. Renal oxidative damage and inflammation were significantly increased in TPEN/diabetic group compared to group with diabetes or TPEN alone, shown by increased protein nitration and lipid oxidation, and infiltrated inflammatory cells in the kidney with increased expression of inflammatory mediators, plasminogen activator inhibitor (PAI)-1 and intercellular adhesion molecule (ICAM)-1. In the TPEN/diabetic group, renal fibrosis was also significantly evident compared to those in TPEN or diabetic group, reflected by increased collagen accumulation in glomerulus messangial area and renal expression of pro-fibrotic mediator connective tissue growth factor. These results indicated that Zn deficiency significantly enhanced diabetes-induced renal oxidative damage and inflammation, resulting in severe renal fibrosis. This study suggests that diabetic patients should prevent their Zn deficiency in order to avoid the acceleration of diabetic renal injury.

PS 757 ROLE OF OXIDATIVE STRESS INDUCED BY CHROMATE IN THE ACTIVATION OF MAPK IN NRK-52E CELLS.

L. Arreola-Mendoza[1, 2], J. L. Reyes[3], E. I. Sanchez[3] and L. M. Del Razo[2]. *[1]Biosciences and Engineering, CIIEMAD-IPN, Mexico D.F., Mexico, [2]Toxicology, Cinvestav-IPN, Mexico D.F., Mexico and [3]Physiology & Biophysics, Cinvestav-IPN, Mexico D.F., Mexico.*

Chromate (Cr6+), the most toxic form of this metal, is widely used in industrial processes and is widespread found as environmental toxicant. Renal toxicity has been described in chromium-exposure and antioxidant administration prevents these deleterious effects. In the biological systems reduction of Cr6+ to Cr3+ results in the formation of reactive intermediates such as reactive oxygen species (ROS) leading to oxidative tissue damage and cellular injury. On the other hand, in lung epithelial cells, ROS are mediators of cell signaling. Cr6+ leads to activation of c-Jun N-terminal kinase (JNK), p38 and extracellular signal regulated kinase (ERK), members of the mitogen-activated protein kinases (MAPKs). In this study we evaluate the role of ROS generation by Cr6+ exposure in the activation of JNK, p38, and ERK1/2 in NRK-52E, cellular line derived from renal proximal tubule. Cells viability was evaluated in a dose-response curve (range of 10 to 200 μM) by trypan blue and lactate dehydrogenase release (LDH). Thereafter, cellular culture (7 days of growing) was exposed to Cr6+ (100 μM) at 5, 10 and 30 min and 1 and 6 h, with or without alpha-tocopherol treatment (25 μM). Phosphorilation of MAPKs was evaluated by Western blotting and ROS level by DCFDA (5', 6' –chloromethyl -2' 7'-dichlorofluorescin diacetate). Cr6+ induced time-depending ERK1/2 activation, as indicated by phosphor-ERK (1/2), from 5 min, p38 phosphorylation was slightly detectable only at 6 hours without change in the activation of JNK. Cr6+ induced ROS generation since 5 min until 1 h, while at 6 h ROS level was as a control. ROS were induced in the cells simultaneously treated with alpha-tocopherol only at 5 and 10 min, and notably in presence of alpha-tocopherol ERK1/2 was not activated. Our results showed a clear relationship between the induction of ROS and the activation of ERK1/2 in NRK-52E. (Funding by CONACYT Mexico)

PS 758 ALUMINUM CITRATE AND COM: MECHANISMS FOR DECREASED RENAL INJURY IN A RAT MODEL OF ETHYLENE GLYCOL POISONING.

L. M. Besenhofer, M. C. McLaren and K. E. McMartin. *Pharmacology, Toxicology, and Neuroscience, Louisiana State University Health Sciences Center - Shreveport, Shreveport, LA.*

Calcium oxalate monohydrate (COM) crystals are responsible for the kidney injury associated with ethylene glycol (EG) exposures. In cases where anti-metabolite therapy is delayed, long-term kidney injury is common and current treatments do not address the mechanism (accumulation of COM). Our *in vitro* studies have shown that aluminum citrate, uniquely among citrate salts, blocks the toxicity in human proximal tubule cells. This study was designed to evaluate the efficacy and mechanism of action of aluminum citrate in a rat model of EG poisoning. Wistar rats were placed into five treatment groups including control (water at time 0), EG (6 g/kg, gavaged at time 0), aluminum citrate, EG + aluminum citrate and EG +

sodium citrate. Citrates (0.2 mmol/kg, saline for controls) were given IV at 6, 12, 24, 36, 48, and 60 h. Urines and plasmas were analyzed for markers of kidney injury and levels of aluminum, citrate, oxalate and calcium. Blood urea nitrogen (BUN) levels in EG + aluminum citrate rats were significantly lower than those in EG + sodium citrate rats, but not different from either EG or control rats. Citrate was decreased in all groups treated with EG compared to the control group, due to the EG-induced metabolic acidosis which promotes citrate reabsorption in the proximal tubules. Urine samples were centrifuged to separate soluble urinary components from insoluble moieties like COM. Urinary aluminum was primarily found in the supernatant and not in the pellet. In contrast, calcium levels were highest in the pellets of rats treated with EG and treatment with aluminum citrate increased calcium excretion in the pellet. Similarly, treatment with EG + aluminum citrate appeared to increase urinary oxalate excretion compared to EG and EG + sodium citrate rats. Taken together, these results suggest that aluminum citrate could decrease COM-induced renal injury by enhancing the excretion of both calcium and oxalate thus decreasing their retention in the kidney.

PS 759 SUB-CHRONIC ETHANOL EXPOSURE INDUCES ACUTE RENAL INJURY AND FIBROSIS IN RATS.

C. Latchoumycandane[1], J. Liu[1], L. E. Nagy[2], A. E. Feldstein[1] and T. M. McIntyre[1]. [1]*Cell Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH and* [2]*Pathobiology and Gastroenterology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH.*

Ethanol exposure induces hepato-renal injury in human and rodent models, and recently we have reported that sub-chronic ethanol exposure increases circulating oxidized phospholipids in rats as well as in alcoholic steatohepatitis patients. However, the biological role of circulating oxidized phospholipids in extra-hepatic tissues remains unclear. Interestingly, exogenously administered fluorescent Platelet activating factor (PAF, a bioactive phospholipid) is predominantly eliminated by kidney, and some oxidized phospholipids have PAF-receptor agonist activity. Based on this, we hypothesized that ethanol induces acute renal injury. In this study we find by LC/MS/MS analysis that rats exposed to a Lieber-DeCarli ethanol diet for 4 weeks significantly increased circulating PAF, peroxidized phophatidylcholine (HpODE-PC), and the pro-apoptotic oxidatively truncated phospholipid azelaoyl-PC derived from HpODE-PC. The expression of inflammatory cytokine Tumor necrosis factor-α and apoptotic active-caspase-3 as well as the number of TUNEL positive cells (apoptotic cells) increased significantly in kidney of ethanol-fed rats as compared with pair-fed. Most strikingly, the renal fibrosis markers, Transforming Growth Factor β-1 and α-smooth muscle actin are increased in ethanol-fed rats, along with collagen IV. Masson trichrome staining of ethanol-fed rat kidney also confirms the presence of collagen fibers in inter tubular space. Kidney injury molecule-1 (KIM-1), a marker for proximal tubular injury has been reported to increase during acute renal injury and we find higher expression of KIM-1 in the proximal tubules of ethanol fed-rats kidneys. The increase in circulating oxidized phospholipids in rats is concordant with all renal injury markers. These results clearly suggest that circulating bioactive oxidized phospholipids correlate with acute renal injury, and development of renal fibrosis. This is the first demonstration of ethanol-induced renal fibrosis.

PS 760 BODY ADIPOSITY AND DIETARY FATTY ACIDS INDUCE CYTOKINE EXPRESSION AND ACTIVATION OF TUMOR PROMOTING PATHWAYS IN THE RAT KIDNEY.

A. Bort[1, 2], G. Ananthakrishnan[1], D. Perez-Tilve[1], D. R. Dietrich[2], M. H. Tschöp[1], P. T. Pfluger[1] and K. Stemmer[1, 2]. [1]*Department of Internal Medicine, University of Cincinnati, Cincinnati, OH and* [2]*Human and Environmental Toxicology, University of Konstanz, Konstanz, Germany.*

Obesity is a major risk factor for numerous co-morbidities, including renal cancer. However, the mechanisms by which obesity increases renal cancer risk are still unclear. Here, we aim to compare the impacts of body adiposity vs. increased dietary fatty acids on renal inflammatory and carcinogenic pathways in rats.
To delineate renal consequences of body adiposity and dietary fatty acids, we selected two different phenotypes from high fat diet fed rats: high susceptibility to diet induced obesity (DIOsens) and partial resistance to diet induced obesity (DIOres) with 56% and 18% overweight compared to a lean chow fed group, respectively. Circulating cytokines, renal cytokine expression and overall pathology were investigated in vivo. Activation of corresponding downstream pathways like tumor promoting mTOR/S6 kinase pathway, were analyzed in vivo and in fatty acid-exposed NRK-52E rat kidney cells.
Similar circulating levels of interleukin 1β and monocyte chemo-attractant protein 1 (MCP1) in DIOres, DIOsens and chow rats did not suggest systemic inflammation. However, a local renal inflammatory reaction, paralleled by an increased expression of interleukin 1β and TNFα was detected in the renal cortex of DIOsens rats, and to lesser extent DIOres rats. Using immunohistochemistry, we detected active mTOR signaling in cortex areas with increased monocyte infiltration and regenerative cell proliferation, which was more pronounced in DIOsens than in DIOres rats. mTOR activation was verified in fatty acid-exposed NRK-52E cells.
In summary, our data suggests that the degree of adiposity in diet-induced obesity, and to lesser extent the excess of dietary fatty acids, are major determinants for a pro-inflammatory and pro-carcinogenic condition in rat kidneys, which might provide an ideal microenvironment for the development of obesity-induced renal cancer.

PS 761 TRANSPORT OF MERCURIC SPECIES IN RABBIT RENAL BRUSH-BORDER MEMBRANE VESICLES.

C. Bridges, L. Joshee and R. K. Zalups. *Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA.*

Cysteine- (Cys) and homocysteine- (Hcy) *S*-conjugates of inorganic mercury (Hg), have been shown to be taken up at the luminal membrane of renal proximal tubular cells. Although one mechanism for this uptake (i.e., amino acid transporter, system $b^{0,+}$) has been identified, it is likely that other carriers are also involved in this process. Therefore, the purpose of the current study was not only to characterize the uptake of Cys- and Hcy-*S*-conjugates of Hg at the brush-border membrane of proximal tubular cells, but also to identify other possible mechanisms that may be involved in the uptake of these conjugates. Brush-border membrane vesicles were isolated from the cortex and outer stripe of the outer medulla from rabbit kidneys as described previously. LLC-PK_1 cells were also used as a control model system. Analyses of saturation kinetics for arginine and phenylalanine indicate that the isolated vesicles are viable models for studies of proximal tubular transport processes. The uptake of each mercuric conjugate was found to be time-dependent and saturable. Addition of Cys or Hcy significantly reduced the uptake of Cys- and Hcy-*S*-conjugates of Hg, respectively. Analyses of substrate specificity using LLC-PK_1 cells showed that various amino acids were able to block the uptake of each conjugate. We conclude that Cys- and Hcy-*S*-conjugates of Hg are taken up at the brush-border membrane of proximal tubular cells by multiple amino acid transporters, including system $b^{0,+}$.

PS 762 KIDNEY PROXIMAL TUBULE-ON-A-CHIP FOR DRUG TRANSPORTER STUDIES AND NEPHROTOXICITY ASSESSMENT.

K. Jang[1, 2], D. Huh[1, 3], G. A. Hamilton[1], A. Bahinski[1], K. Suh[2] and D. E. Ingber[1, 3, 4]. [1]*Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA,* [2]*Interdisciplinary Program in Nano-Science and Technology, Seoul National University, Seoul, Republic of Korea,* [3]*Vascular Biology Program, Departments of Pathology and Surgery, Children's Hospital, Boston, MA and* [4]*School of Engineering and Applied Sciences, Harvard University, Cambridge, MA.*

There is a great need for more predictive in vitro human kidney models for investigating absorption, distribution, metabolism, excretion, and toxicological properties of new chemical entities during the drug development process. Here we describe a biomimetic microsystem that reconstitutes critical functional aspects of renal proximal tubule of the human kidney. In this system, kidney tubular epithelial cells are cultured on a porous flexible matrix-coated membrane within a microfluidic system and exposed to cyclic mechanical strain and fluid dynamic forces that mimic the in vivo microenvironment. Culture of primary human proximal tubule cells under a fluid shear stress similar to that of normal human proximal tubule (~ 0.2 to 5 dyn/cm2) resulted in enhanced cell polarization, cytoskeletal reorganization, and molecular transport in response to hormonal stimulation. Renal drug transporter expression, specific markers of proximal tubule function, and effects of known nephrotoxic compounds were also analyzed using this microdevice, which reproduces complex physiological responses to hormonal stimulation, molecular transport, and metabolic activities of the renal tubule. The results obtained in this investigation suggest that this novel system may provide a useful and cost-effective tool for studying biotransformation profiles, renal pharmacology, renal drug transport and toxicity relevant to the human kidney, and hence help to facilitate the drug development process.

PS 763 ATTENUATION OF OCHRATOXIN A TOXICITY BY INHIBITION OF ORGANIC ANION TRANSPORT.

A. Sarma and S. M. Ford. *Toxicology Program, St. Johns University, Queens, NY.*

Ochratoxin A (OTxA) is a nephrotoxic mycotoxin which is transported in the renal proximal tubule by organic anion transporters (OATs). OAT1 and OAT3 on the basolateral membrane contribute to OTxA secretion into tubular fluid. The re-

cently characterized transporter OAT5 is located on the apical membrane and may effect reabsorption of OTxA. These uptake transporters are thought to contribute to the toxicity of OTxA. Our hypothesis was that OTxA toxicity would be attenuated by the OAT inhibitor probenecid (PROB) or the OAT5 substrate dihydroepiandrosterone sulfate (DHEAS). LLC-PK1 cells were grown to confluence in 35 mm tissue culture plates and treated for 24 hr with varying concentrations of OTxA or DHEAS. Viability was assessed by trypan blue exclusion. LC50s determined for OTxA and DHEAS and the LC20 for DHEAS were used, as well as a non-toxic concentration of PROB. Cultures were treated for 24 hr with OTxA (3 μM), DHEAS (5.5 or 55 μM), PROB (0.5 mM), OTxA + DHEAS, or OTxA + PROB. The results were expressed as % viable control (%VC), defined as: [(# live cells treated group)/(# live cells control group)] x 100. The %VC of cells treated with OTxA alone was 57.9±5.4%. For DHEAS-treated cells (55 μM) the %VC was 52.2±1.5%, for treatment with 5.5 μM DHEAS (n=2), 79.3% and that of PROB-treated cells, 90.8±3.1%. Simultaneous treatment with OTxA + DHEAS (55 μM) resulted in significantly decreased viability (31.2±0.3%), whereas the viability of cells treated with PROB + OTxA (81.3±1.6%) was significantly higher than OTxA alone ($p<0.05$). Viability of cells treated with OTxA + 5.5 μM DHEAS was slightly higher as well. Attenuation of toxicity by PROB demonstrates that transport of OTxA into cells by contributes to its toxicity. Probenecid inhibits both the basolateral and apical OATs and further work is needed to resolve the contributions of the individual OATs to OTxA toxicity.

PS 764 EXPRESSION OF THE ZINC/CADMIUM TRANSPORTER, SCL39A8 (ZIP8), IN NORMAL AND TRANSFORMED CELLS OF THE PROSTATE AND BLADDER, AND IN RENAL CORTICAL TISSUE AND DERIVED CELL CULTURE.

A. Ajjimaporn, S. H. Garrett, S. Somji, X. Zhou, S. Satarug, M. Sens and D. A. Sens. *Pathology, University of North Dakota, Grand Forks, ND.*

The heavy metal cadmium (Cd^{+2}) has been implicated in renal toxicity and in the formation of bladder and prostate tumors. Recently, the zinc family ZIP8 transporter has been identified as participating in cadmium transport and conferring susceptibility of the testes to the toxic effects of cadmium. This transporter has also been found in kidney where increased expression also confers susceptibility to cadmium toxicity. ZIP8 expression levels were measured whole kidney cortical tissue and cultures of human epithelial cells exhibiting properties of proximal tubules, using immunohistochemistry, RT-PCR, and western analysis. Expression was detected in the kidney using all three techniques, with immunohistochemistry staining proximal and distal tubules whereas glumeruli where negative. Expression was also assessed in normal bladder UROtsa cells and in Cd^{+2} and As^{+3} transformed UROtsa cell isolates. The levels of ZIP8 expression were higher in most of the metal transformed UROtsa cell isolates, as well as the corresponding heterotransplant tumors in nude mice. Normal human urothelium showed strong staining for ZIP8 expression, indicating this transporter may function in Cd^{+2} uptake during environmental exposure. The levels of this transporter were also assessed in prostate where the normal-non-tumorigenic cell line, RWPE-1, exhibited low levels of ZIP8 expression and the three human prostate tumor cell lines, PC-3, LNCaP, and DU-145, showed considerable increases in expression. Normal prostate tissue exhibited low levels of ZIP8 expression. These data indicate that the ZIP8 zinc transporter may have significant regulation in many tumor cell types and that those tissues that exhibit cadmium-induced transformation also show expression of a transporter capable of facilitating accumulation of this carcinogenic metal.

PS 765 FIBRINOGEN IS SYNTHESIZED BY THE KIDNEY AND MODULATES TISSUE REPAIR FOLLOWING RENAL INJURY.

A. Krishnamoorthy[1], A. K. Ajay[1], D. Hoffmann[1], T. Kim[2], S. Selvakumar[1], V. Ramirez[1], N. Bobadilla[3], P. Park[2] and V. S. Vaidya[1]. [1]*Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA,* [2]*Center for Biomedical Informatics, Harvard Medical School, Boston, MA and* [3]*National Autonomous University of Mexico, Mexico City, Mexico.*

Fibrinogen (Fg) is a soluble 340KDa dimeric glycoprotein made of three distinct pairs of polypeptide chains (Fgα, Fgβ and Fgγ). In genome-wide expression analysis of rat kidney following 20 minutes of bilateral ischemia/reperfusion (I/R) injury we found Fgβ to be the second highest gene up regulated amongst 22,523 genes at 24 hours. Fg has been recognized as an important regulator of hemostasis, inflammation and wound healing but little is known about the functional significance of Fg signaling in the kidney. We hypothesized that kidney injury up regulates Fg, triggering tissue repair. Real time PCR data confirmed that Fgβ increased significantly (~60 fold) in medulla while mRNA levels of Fgα and Fgγ ranged from 3 fold in cortex up to 18 fold in medulla over a period of 5 days following reperfusion. Protein levels of the three chains peaked at 24 and remained elevated above baseline by 120 h following reperfusion. Significant elevations in mRNA of Fgα (~30 fold), Fgβ (~30 fold) and Fgγ (~250 fold) were also observed in mice on day 3 following 20 mg/kg cisplatin administration ip. Immunostained rat and human kidney tissues revealed that under normal conditions Fgα is expressed by epithelial cells of kidneys and is up regulated following injury, over expression of Fgβ is predominantly in the renal interstitium and Fgγ relocalizes from basolateral to apical membrane. We also observed up regulation of IL-6 and activation of p-Stat-3 following injury, potentially binding to the Fgβ promoter, thereby regulating its transcription. Furthermore, Fg protein (0.5, 1 or 2 mg/ml) stimulated proliferation of kidney epithelial cells (HEK293, LLCPK1, and MDCK) by 180 % in a dose dependent manner. Thus, our results demonstrate that fibrinogen is synthesized by the kidney and potentially plays an important role in modulating tissue repair.

PS 766 ETHANOL(ETOH)EXPOSURE *IN VIVO* POTENTIATES DICLOFENAC(DCLF)-INDUCED NEPHROTOXICITY BY DESTABILIZING GENOMIC INTEGRITY AND PRO- AND ANTIAPOPTOTIC GENES.

S. D. Pravasi, S. N. Desai and S. D. Ray. *Molecular Toxicology Labs, Division of Pharmaceutical Sciences, AMS College of Pharmacology & Health Sciences, Brooklyn, NY.*

More than 70 million prescriptions for NSAIDs are written each year, and over-the-counter use included, more than 30 billion doses of NSAIDs are consumed annually in the US alone. In 2007, Poison Control Centers received 307,590 calls with NSAIDs involved; of these, 205,245 cases were found to be due to single exposures. Among several NSAIDs, DCLF is frequently prescribed for pain management and treatment of various conditions like arthritis, rheumatoid arthritis and osteoarthritis. Our previous studies have shown nephrotoxic and apoptogenic potentials of DCLF (Hickey et al., FRBM, 2001; Bhatt et al., FASEB J, 2008). This study investigated: i) whether exposure to subtoxic doses of ethanol can potentiate DCLF-induced nephrotoxicity, and ii) whether the underlying mechanisms of this potentiation are genomic injury, increased apoptosis and an imbalance in pro- and anti-apoptotic gene expression. To explore this interaction, female ICR mice (3 mo old) were kept on regular diet and, either plain tap water or EtOH containing drinking water (Wk1:3%, Wk2:4%, Wk3-5%, Wk-6%) for 4 weeks. On day-28 a single dose of DCLF (150 mg/kg,ip) was administered, and all animals were sacrificed 24hours later. Blood was collected for serum chemistry and kidneys for tissue biochemistry & pathology. Serum BUN reflected potentiation of nephrotoxicity (mg/dl: Con:30; DCLF150:68; EtOH:31; EtOH+DCLF:128). Concurrent genomic injury and oxidative stress caused genomic instability and escalated kidney DNA fragmentation (2.7 fold) and pro-apoptotic Bax/Bad expression in EtOH+DNA group compared to DCLF alone. In contrast, Bcl-XL expression considerably declined in EtOH+DCLF group compared to DCLF-alone. Kidney sections mirrored cell death patterns (apoptosis & necrosis) that were consistent with tissue biochemistry in all treatment groups. Overall, this study suggests that EtOH-exposure prior to DCLF administration may exacerbate nephrotoxicity and DCLF prescription to regular drinkers may require precaution.

PS 767 DIVERGENT ASSOCIATIONS OF BLOOD AND URINE CADMIUM LEVELS WITH KIDNEY FUNCTION.

V. Weaver[1, 2], M. Tellez-Plaza[1], A. Navas-Acien[1], B. Jaar[1, 2], J. Fadrowski[2], P. Muntner[4], E. Silbergeld[1] and E. Guallar[1, 2, 3]. [1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,* [2]*Johns Hopkins University School of Medicine, Baltimore, MD,* [3]*Centro Nacional de Investigaciones Cardiovasculares School of Medicine, Madrid, Spain and* [4]*University of Alabama, Birmingham, AL.*

Cadmium is an established nephrotoxicant at high exposure levels. Environmental exposure at lower levels is widespread but data on kidney impact at these levels are scarce. We reported an association between higher blood cadmium levels and reduced estimated glomerular filtration rate (eGFR; <60 mL/min/1.73 m2) in adults who participated in the 1999-2006 National Health and Nutrition Examination Survey (NHANES) (Navas-Acien et al., Am J Epidemiol. 2009). Recently, we compared associations of blood and urine cadmium with reduced eGFR in the subset of 5,770 1999-2008 NHANES participants aged ≥ 20 years in whom both urine and blood cadmium were measured. Geometric mean blood and urine cadmium and

MDRD eGFR levels were 0.40 μg/L, 0.27 μg/g creatinine, and 86.8 ml/min/1.73 m2, respectively. After adjustment for survey year, sociodemographic factors, chronic kidney disease risk factors and blood lead, the odds ratio (95% CI) for reduced eGFR comparing the 75th to the 25th percentile of blood cadmium levels was 1.37 (1.09, 1.72), consistent with findings in the larger population. In contrast, for urine cadmium, after adjustment for urine creatinine and the above co-variates, the corresponding odds ratio (95% CI) was 0.46 (0.32, 0.66). Without adjustment for urine creatinine, the odds ratio was 0.96 (0.79, 1.16). The reason for these inconsistent associations is unclear. Potential explanations for these unexpected findings include the impact of adjusting the association of urine cadmium with kidney outcomes for urine dilution with urine creatinine which is significantly associated with eGFR in this population; an impact of renal filtration on cadmium excretion; and cadmium-related hyperfiltration with cumulative cadmium dose. Prospective epidemiologic studies and experimental studies are needed to understand these cross-sectional findings.

PS 768 PARADOXICAL ASSOCIATIONS OF URINE CADMIUM WITH SERUM CREATININE BUT NOT CYSTATIN-C-BASED eGFR.

J. Fadrowski[1], N. Kim[3], B. Jaar[1, 2], J. Spector[4], P. Parsons[5], A. Steuerwald[5], A. Todd[6], D. Simon[7], B. Lee[3], B. Schwartz[1, 2] and V. Weaver[1, 2]. *[1]Johns Hopkins University School of Medicine, Baltimore, MD, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, [3]SoonChunHyang University, Asan, Republic of Korea, [4]University of Washington, Seattle, WA, [5]New York State Department of Health, Albany, NY, [6]Mount Sinai School of Medicine, New York, NY and [7]Biostatistical Consulting, Cincinnati, OH.*

We recently reported associations between higher urine cadmium and higher creatinine-based estimates of kidney function in an occupationally lead exposed population (Weaver et al., Occ Env Med, In press). One potential explanation for these unexpected results is that cadmium adjustment for urine dilution with urine creatinine may alter associations with kidney outcomes due to the positive association between urine and serum creatinine observed in this population. Therefore, we evaluated associations of urinary cadmium with an alternate marker of kidney function, serum cystatin C, in this population. In 712 lead workers, mean (SD) urine cadmium and estimated glomerular filtration rate (eGFR) using a multi-variable age, sex, race, and cystatin C equation (Stevens et al., Am J Kidney Dis. 2008) were 1.15 (0.66) μg/g creatinine, and 112.0 (17.7) ml/min/1.73 m^2, respectively. After adjustment for sociodemographic and kidney disease risk factors and lead dose, ln-urine cadmium was not associated with cystatin C or cystatin-C-based eGFR. However, similar to results with the creatinine-based MDRD eGFR equation, higher ln-urine cadmium was positively associated with GFR estimated with an equation incorporating both serum cystatin C and creatinine (β = 4.1 ml/min/1.73 m^2; 95% CI =1.6, 6.6). These results suggest that use of urine creatinine to adjust biomarkers for urine dilution may impact associations with serum creatinine-based kidney outcome measures. However, several factors have recently been shown to have opposing effects on creatinine and cystatin C; cadmium exposure may be another.

PS 769 TISSUE DEPOSIT AND TOXIC MECHANISM OF CADMIUM IN THE KIDNEY OF FE-DEPLETED RATS.

J. Park[1], D. Kim[1], H. Bae[1], T. Lee[2] and B. Choi[1]. *[1]Preventive Medicine, Chung-Ang University, Seoul, Republic of Korea and [2]Pathology, Chung-Ang University, Seoul, Republic of Korea.*

Cadmium (Cd), which has been known as a human carcinogen, is widely distributed in the environment. General population are exposed to the cadmium through the contaminated water and diet. In this study, we used the Fe-depleted animal model to investigate the absorption, deposition, toxicity and it's mechanism, and interaction with Fe in the kidney, which is a target organ of cadmium, of Cd-exposed animal through a drinking water. Cadmium, 0, 10, 100 mg Cd/L in drinking water, was administered to Fe-deficient or Fe-sufficient animals, respectively, for 4 or 8 weeks. The concentration of Cd were increased with a dose-dependent pattern in the kidney, which were higher in Fe-deficient rats than in Fe-sufficient animals. The levels of NAG activity, RBP, MDA in urine were increased after Cd administration, which findings were aggravated in Fe-deficient animals. The increase of BUN in serum and abnormal morphological findings in the kidney were observed in high-dose Cd administerd Fe-deficient animals. In summary, repeated Cd exposure through drinking water produce kidney toxicity, which might be caused by oxidative stress mechanism. Depletion of body Fe is more susceptible to the Cd toxicity by increasing of Cd absorption in the kidney.

PS 770 CADMIUM CAUSES OVER-ACCUMULATION OF P53 THROUGH THE SUPPRESSION OF GENE EXPRESSION OF UBE2D FAMILY IN RAT PROXIMAL TUBULE CELLS.

M. Tokumoto[1, 2], Y. Fujiwara[2], T. Hasegawa[3], Y. Seko[3], H. Nagase[1] and M. Satoh[2]. *[1]Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University, Gifu, Japan, [2]Laboratory of Pharmaceutical Health Science, School of Pharmacy, Aichi Gakuin University, Nagoya, Japan and [3]Department of Environmental Biochemistry, Yamanashi Institute of Environmental Sciences, Fujiyoshida, Japan.*

Using microarray analysis, we have recently found that the gene expression of Ube2d4, which is a ubiquitin-conjugating enzyme and one of Ube2d family, is suppressed by cadmium (Cd) under non-toxic condition in rat proximal tubule cells (NRK-52E cells). In this study, to clarify the involvement of Ube2d family against Cd cytotoxicity, we examined the effect of Cd on the gene expression of Ube2d family and the cellular accumulation of p53 protein, which is ubiquitinated by Ube2d family, using NRK-52E cells. NRK-52E cells were treated with 5 μM Cd for 3 to 24 h. The activity of lactate dehydrogenase leaked into the conditioned medium from the cells was significantly elevated by Cd treatment for 24 h, but not for 12 h. The p53 protein level significantly increased before appearance of the cytotoxicity in NRK-52E cells treated with Cd. Moreover, mRNA levels of Ube2d1, Ube2d2, Ube2d3 and Ube2d4, which are Ube2d family, were remarkably decreased before appearance of the overaccumulation of p53 by Cd treatment. On the other hand, Cd did not affect either p53 mRNA level or proteasome activity. These results suggest that overaccumulation of p53 by Cd may relate to the development of Cd cytotoxicity and may be due to the suppression of p53 degradation through the inhibition of gene expression of Ube2d family without the inhibition of proteasome.

PS 771 CHANGES TO CONNEXIN EXPRESSION IN METALLOTHIONEIN-3 POSITIVE AND NEGATIVE HUMAN PROXIMAL TUBULE CELLS AFTER CADMIUM TREATMENT.

L. C. Burgess, T. E. Bhobho, J. R. Murray and M. C. McCoy. *Natural Sciences, Dickinson State University, Dickinson, ND.*

Gap junction communication allows direct transfer of ions and molecules of up to 1,500 Daltons between cells to maintain electrical and metabolic homeostasis. Gap junctions are made of connexins (Cx) consisting of six individual transmembrane proteins. There are 20 different Cx genes in humans. Cx genes act as tumor-suppressors by maintaining homeostatic control in multicellular organisms. MT-3 is the third isoform of metallothionein, and is a major heavy metal binding protein involved in cadmium nephrotoxicity. It is thought that MT-3 affects Cx expression like it does with vectorial active ion transport. Cadmium at even low exposures alters renal tubule function and MT-3 which is expressed in human proximal tubule cells, plays a protective role against cadmium toxicity. An immortalized model of these cells without MT-3 expression (HK-2) and a stable transfection of MT-3 into the HK-2 cells (HK-2MT-3) were used to compare cadmium treatments and MT-3 gene to changes in Cx expression. Standard RT-PCR showed that Cx 26, 30.2, 30.3, 32, 43, 45, and 58 were expressed in both cell lines. The relative expression levels of the Cx were determined using Reverse Transcriptase Real-Time PCR which showed a significant expression of the Cx proteins 26, 30.3, 32, 43 and 45. The HK-2MT-3 cells showed increased expression of Cx 26, 30.3, and 45 over the HK-2 cells and the HK-2 had an increased expression of Cx 32 and 43. Cx 30.2 and 58 showed no difference in either cell line. The MTT assay showed no significant toxicity from CdCl2 treatments below 10μM and 4 hr or less, therefore, a dose range of 0 to 10μM was used. Cx 32 and 43 showed decreases in expression by 4hr at all doses in both cell lines. Cx 45 increased expression at the 2.5 and 5μM and decreased at the 10 μM dose at 4 hr. The expression was strongest in Cx 43 then 45 and lowest in 32. The MT-3 gene only increased expression of Cx 45. This study shows that cadmium generally decreases Cx expression with some exceptions. The MT-3 gene may have limited effect on Cx expression.

PS 772 STATISTICAL ANALYSIS OF LONGITUDINAL GENE EXPRESSION PROFILES IN HUMAN PROXIMAL TUBULE CELLS EXPOSED TO CADMIUM.

S. H. Garrett, K. Clarke, D. A. Sens, S. Somji, M. Sens and K. Zhang. *Pathology, University of North Dakota, Grand Forks, ND.*

Cadmium (Cd^{2+}) is a known nephrotoxin causing tubular necrosis during acute exposure and potentially contributing to renal failure in chronic long-term exposure. To investigate changes in global gene expression elicited by cadmium, an *in-vitro*

exposure system was developed from cultures of human renal epithelial cells derived from cortical tissue obtained from nephrectomies. These cultures exhibit many of the qualities of proximal tubule cells. Using these cells, a study was performed to determine the cadmium-induced global gene expression changes after short-term (1 day, 9, 27, and 45 μM) and long-term cadmium exposure (13 days, 4.5, 9, and 27 μM). These studies revealed fundamental differences in the types of genes expressed during each of these time points. The obtained data was further analyzed using regression and mutual information analysis to identify genes with a high probability of mutual dependence. Regression analysis showed 1720 genes were induced and 2316 genes were repressed by Cd^{2+} within 1 day, and 1523 and 2146 genes were induced and repressed, respectively, after 13 days. A network model based on mutual information was used to derive an interaction map of these genes. The most prominent network consisted of IL13RA2, DNAJA4, HSPA6, AKAP12, ZFAND2A, AKR1B10, SCL7A11, and AKR1C1.

PS 773 ASSOCIATIONS OF MULTIPLE METALS WITH KIDNEY OUTCOMES IN LEAD WORKERS.

R. L. Shelley[1], N. Kim[2], P. J. Parsons[3], J. Agnew[1], A. J. Steuerwald[3], B. G. Jaar[1], B. Lee[2], B. S. Schwartz[1], G. Matanoski[1], A. C. Todd[4], D. Simon[5] and V. Weaver[1]. *[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, [2]SoonChunHyang University, Asan, Republic of Korea, [3]New York State Department of Health, Albany, NY, [4]Mount Sinai School of Medicine, New York, NY and [5]Biostatistical Consulting, Cincinnati, OH.*

Environmental exposure to metals is widespread. Several are potential nephrotoxicants yet few studies have examined the impact of co-exposure to these metals in workers with occupational exposure to nephrotoxicants such as lead. In analyses in an occupationally lead exposed population to address this gap, we unexpectedly found that environmental urine cadmium (Cd) was positively associated with estimates of glomerular filtration rate [eGFR]) (Weaver et al., OEM. In press). We also evaluated associations of urine antimony (Sb). In 684 lead workers, mean (SD) blood and tibia lead; urine Sb and Cd; and eGFR using the Modification of Diet in Renal Disease equation were 23.2 (14.3) μg/dl, 27.1 (29.3) μg Pb/g bone mineral, 2.4 (7.6) μg/g creatinine, 1.1 (0.6) μg/g creatinine, and 97.7 (19.4) ml/min/1.73 m^2, respectively. After adjustment for lead dose, urine creatinine, and sociodemographic and kidney disease risk factors, higher ln-urine Sb was associated with lower serum creatinine and higher eGFR (β = 1.5 ml/min/1.73 m^2; 95% CI = 0.5, 2.5) and ln-N-acetyl-β-D-glucosaminidase (NAG), a kidney biomarker. After additional adjustment for ln-Cd, Sb remained associated with serum creatinine and eGFR (β = 1.2 ml/min/1.73 m^2; 95% CI = 0.2, 2.2) but not NAG. Paradoxical associations with both Sb and Cd suggest that the mechanism for these findings may impact a wider range of urine biomarkers in kidney function research and support the need to determine that mechanism. These results also highlight the potential for environmental level Cd exposure to confound associations of other metals with NAG.

PS 774 GENDER DIFFERENCES IN THE MOLECULAR MECHANISMS OF BROMATE INDUCED NEPHROTOXICITY IN F344 RATS.

N. Kolisetty[1], D. A. Delker[2], C. Nelson[2], S. Muralidhara[1], R. J. Bull[3], O. Quinones[4], G. Zhongxian[5], S. Snyder[4], J. Cotruvo[6], J. W. Fisher[7], C. Ong[8] and B. S. Cummings[1]. *[1]University of Georgia, Athens, GA, [2]University of Utah, Salt Lake City, UT, [3], Mo Bull Consulting, Richland, WA, [4]Water Quality Research and Development, Henderson, NV, [5]Center for Advanced Water Technology, Singapore, Singapore, [6]Joseph Cotruvo & Associates, LLC, Washington, DC, [7]University of Georgia, Athens, GA and [8]National University of Singapore, Singapore, Singapore.*

The disposition of bromate after oral exposures is similar in male and female rats, which contrast to its ability to preferentially induce nephrotoxicity in male rats. This might be due to gender differences in bromate-induced kidney injury manifested by different gene expression profiles in male and female rats after exposure to bromate. Previous studies using cDNA microarray analysis supports this hypothesis, but additional studies of mRNA and protein changes are needed to confirm the microarray results. Thus, qPCR and immunohistochemistry were used to study expression of several proteins in kidney, thyroid and testicular tissues isolated from male and female (kidney and thyroid only) rats orally exposed to bromate (125 and 400 mg/kg KBrO3) for 28 days. In agreement with the cDNA microarray data, p21 mRNA and protein expression increased in kidney and thyroid in both male and female rats in a dose-dependent manner. Similar trend was observed in testicular tissue. In contrast to the cDNA microarray data, protein expression of kidney injury molecule 1 occurred earlier and at higher levels in female kidneys. The protein expression of clusterin, an anti apoptotic protein, was increased in a dose-dependent manner in male rat kidneys correlating with increases in mRNA transcript levels and in, thyroid, and testes, but not in female kidneys or thyroid. Increases in expression of clusterin and p21, correlated to cell proliferation and DNA damage as measured by BrdU and 8-OH-dG staining, respectively. To our knowledge, these data are the first to demonstrate that bromate exposure increases clusterin, and to suggest that the gender-specific nephrotoxicity induced by bromate correlate to clusterin, but not p21, expression.

PS 775 NKTR-181, A NOVEL POLYMER CONJUGATED OPIOID AGONIST, DEMONSTRATES REDUCED TOXICITY AND CNS SIDE EFFECTS RELATIVE TO OXYCODONE.

E. Tonkin, S. Wong, A. Odinecs and T. D. Sweeney. *Nektar Therapeutics, San Carlos, CA.*

NKTR-181 is a novel orally available mu-opioid agonist that uses Nektar's advanced polymer conjugate technology to achieve slower kinetics of entry into the brain relative to other opioids, while still producing potent analgesia in mouse models. The goal of these studies was to compare the toxicity of NKTR-181 to that of oxycodone in female rats. Rats (n = 3) were given single oral doses of 0, 200, 500, or 700 mg/kg oxycodone or NKTR-181 and were monitored for changes in clinical condition for 24 hrs. At ≥200 mg/kg, oxycodone produced hypoactivity, hematuria, and mortality, with increased severity of changes and altered respiratory rates at the higher doses. NKTR-181 produced clinical changes only at 700 mg/kg, consisting primarily of hypoactivity. In a subsequent study, rats (n = 3) were given 5 daily doses of NKTR-181 (0, 200, 500, and 1000 mg/kg) or oxycodone (100 mg/kg). Clinical condition and body weight were monitored and samples were collected for toxicokinetic and histopathology assessment. Animals given 100 mg/kg/day oxycodone exhibited hypoactivity, increased muscle tone, behavioral changes such as excessive paw chewing, and decreased body weight compared to controls. For NKTR-181, no behavioral changes were noted at 200 mg/kg, while animals given 500 mg/kg NKTR-181 displayed increased activity and paw chewing, and animals given 1000 mg/kg/day NKTR-181 displayed hypoactivity, altered muscle tone, and mortality (1/3 animals). For 100 mg/kg oxycodone, mild histopathological changes were noted in the urinary bladder, intestinal epithelium, and reproductive and lymphoid tissues. Similar changes were noted at 1000 mg/kg NKTR-181, with mild intestinal changes noted at all doses of NKTR-181. Toxicokinetic assessment demonstrated that NKTR-181 had higher plasma exposure relative to oxycodone on a dose normalized basis. In conclusion, NKTR-181 produces fewer CNS side effects and is tolerated at higher doses than oxycodone. Results from on-going definitive toxicity and safety pharmacology studies with NKTR-181 support these conclusions.

PS 776 RELATIVE TOXICITY ANALYSIS OF DL11F, A HER3, AND EGFR DUAL ACTION ANTIBODY, AND CETUXIMAB IN CYNOMOLGUS MONKEYS.

R. Prell[1], D. Danilenko[1], C. Wang[1], A. Kamath[1], G. Schaefer[1], P. Allen[2], J. Roberts[2] and W. Halpern[1]. *[1]Genentech, South San Francisco, CA and [2]Valley Biosystems, West Sacramento, CA.*

DL11f is an engineered monoclonal antibody in which each of the two antigen binding fragments (Fabs) is capable of binding to HER3 and EGFR with high affinity. DL11f blocks ligand binding to both receptors and demonstrates equivalent or superior efficacy to other anti-EGFR mAbs in mouse xenograft models. EGFR and HER3 are members of the epidermal growth factor receptor family and deregulation of EGFR and HER3 signaling have been implicated in tumorigenesis and tumor progression. Though EGFR is a clinically validated target, inhibition is associated with dermatologic toxicity in up to 90% of patients and can become severe enough to require dose-reduction or discontinuation. To determine the relative dermatologic toxicity of DL11f compared to cetuximab, a pilot toxicity study was performed in female cynomolgus monkeys. Animals (3/group) were dosed IV weekly for 5 weeks with cetuximab at 25 mg/kg, or DL11f at 25 mg/kg or 12.5 mg/kg. Dermatologic toxicity was observed between the 3rd and 4th doses in all three animals dosed with cetuximab, consistent with prior reports. Gross lesions were characterized by inguinal, axillary, and chest erythema and exfoliation. At necropsy 4 weeks after the final dose, there was still histopathologic evidence of minimal to mild perivascular dermal inflammation, acanthosis with orthokeratotic hyperkeratosis, and occasional intraepidermal edema. In contrast, only 1 of 3 animals dosed with DL11f at 25 mg/kg had evidence of dermatologic toxicity that was of lesser severity, compared to cetuximab treatment, and appeared after the 6th dose. No animals dosed with 12.5 mg/kg DL11f showed gross evidence of dermatologic toxicity. TK profiles confirmed comparable exposure between animals dosed with cetuximab and DL11f at 25 mg/kg; thus the reduced toxicity was not due to differential drug exposure. These data suggest that the dermatologic toxicity of DL11f is substantially less than that of cetuximab and that DL11f may have a superior clinical safety profile.

PS 777 NONCLINICAL CARCINOGENICITY ASSESSMENT OF PRASUGREL.

L. A. Buckley[2], A. Sanbuissho[1], J. J. Starling[2], M. Knadler[2] and J. A. Jakubowski[2]. [1]*Daiichi Sankyo Co., Ltd., Tokyo, Japan and* [2]*Eli Lilly & Co., Indianapolis, IN.*

Prasugrel, a thienopyridine ADP receptor antagonist, is an orally administered prodrug requiring in vivo metabolism to form the active metabolite that irreversibly inhibits platelet activation and aggregation mediated by the P2Y12 receptor. A comprehensive nonclinical safety assessment including genotoxicity and carcinogenicity studies supported the chronic use of prasugrel in patients with atherothrombotic disease. A special nonclinical carcinogenic risk assessment was undertaken to address FDA-specific concerns relating to the potential for increases in human cancers. Prasugrel was negative in a battery of genotoxicity tests and was not oncogenic in a 2-year rat carcinogenicity study. In a 2-year mouse study, an increase in hepatocellular adenomas was observed; this effect was considered secondary to enzyme induction and not relevant to human safety. Further, the absence of any increase in common background tumors at any other organ site in either rodent study indicated a lack of tumor promoting activity (apart from the CYP450 induction-related increase in mouse liver tumors). Several in vitro and in vivo tumor progression studies provided further support that prasugrel is not a tumor promoter. Specifically, cell culture studies with 3 human tumor cell lines (lung, colon, and prostate) demonstrated that exposure of serum-starved cells to prasugrel's active and major circulating human metabolites does not increase cell proliferation relative to starved cells stimulated to proliferate by the addition of 10% FBS. In vivo studies with nude mice implanted with these 3 human tumor cells also demonstrated that daily dosing of prasugrel does not increase tumor growth relative to vehicle control groups despite levels of active metabolite up to 34-fold higher than exposures achieved at steady state in patients receiving 10 mg maintenance doses. In summary, traditional genotoxicity and 2-year bioassay studies as well as specially designed tumor promotion studies in mouse xenograft models clearly demonstrated prasugrel's lack of tumorigenic potential.

PS 778 OFF-TARGET PLATELET ACTIVATION IN MACAQUES BY A THERAPEUTIC MONOCLONAL ANTIBODY.

M. J. Santostefano[1], N. E. Everds[2], H. M. Vargas[3], M. Fort[4], P. Narayanan[4], D. Tran[2], W. Pan[5], J. Kirchner[6], C. Vissinga[6] and G. S. Elliott[7]. [1]*CBSS - Toxicology, Amgen, Seattle, WA,* [2]*CBSS - Pathology, Amgen, Seattle, WA,* [3]*CBSS - Investigative Toxicology, Amgen, Thousand Oaks, CA,* [4]*CBSS - Investigative Toxicology, Amgen, Seattle, WA,* [5]*Pharmacokinetics and Drug Metabolism, Amgen, Seattle, WA,* [6]*Inflammation Research, Amgen, Seattle, WA and* [7]*Clinical Pathology, Charles River Laboratories, Reno, NV.*

AMGX is a fully human neutralizing IgG2 monoclonal antibody with clinical applications in autoimmune and inflammatory diseases. Dosing with ≥100 mg/kg IV in cynomolgus monkeys resulted in rapid profound thrombocytopenia with decreased platelet granularity, lowered mean arterial pressure, and transient loss of consciousness and flushing. In contrast, none of these effects were observed after 100 mg/kg IV treatment with any of three other neutralizing monoclonal antibodies against the same pharmacological target, indicating that the in vivo findings were specific to AMGX. In vitro, AMGX induced platelet activation in whole blood and platelet-rich plasma from cynomolgus (M. fascicularis), pigtail (M. nemestrina) or rhesus (M. mulatta) macaque species, but not from human (H. sapiens) or baboon (P. c. anubis), as assessed by both aggregometry and surface expression of CD62P and PAC-1. These results were also specific to AMGX since none of the other 3 neutralizing monoclonal antibodies directed against the same target were able to induce in vitro platelet activation in any of the species tested. Together, these data demonstrate that AMGX mediated activation of platelets in vivo and in vitro through an off-target mechanism in a manner that was specific to macaques.

PS 779 CINACALCET HCL: 6-MONTH ORAL TOXICITY STUDY IN JUVENILE BEAGLE DOGS.

K. Black[1], C. Dean[1], N. Everds[1], E. Lesage[2] and M. Loomis[1]. [1]*Amgen Inc., Thousand Oaks, CA and* [2]*Charles River Laboratories Preclinical Services, Senneville, QC, Canada.*

Cinacalcet HCl is a calcimimetic agent that is approved for the treatment of secondary hyperparathyroidism in adult patients with chronic kidney disease receiving dialysis. To support pediatric use, a 6-month oral toxicity study was conducted in juvenile beagle dogs. Male and females (8/sex/group; 10 weeks of age) received cinacalcet HCl by gavage at dose levels of 0, 10, 30 or 100 mg/kg/day for 6 months, following which 4/sex/group were euthanized and necropsies conducted. The remaining animals entered a 3-month treatment-free phase. Emesis, tremors and decreased activity and decreased food consumption occurred at all dose levels. Decreased body weight gain occurred at ≥30 mg/kg. During week 10, dosing was suspended for 7 days for some females that received ≥30 mg/kg to allow them to regain appetite and body weight, and 1 of these animals from the 100-mg/kg group was euthanized in week 13 due to failure to recover. Pharmacologically-mediated decreases in serum ionized calcium occurred at all dose levels. Decreased red cell mass and increased platelets occurred at ≥30 mg/kg. Nonadverse histologic findings consisted of minimal growth plate thickening and several lymphoid changes in the thoracic cavity (lymphoid hyperplasia and mononuclear cell infiltration in the esophagus). There were no treatment-related histologic changes in the heart or reproductive tract tissues. Slight decreases in bone densitometry and geometry parameters occurred at ≥30 mg/kg. All of the changes were fully to partially reversible during the treatment-free phase. Based on the clinical intolerance and growth effects, eg, decreased body weight gain, noted at ≥30 mg/kg, the no-adverse-effect-level in this study was 10 mg/kg. Most of the changes observed in the study were either relatively minor, consistent with the pharmacologic effects of cinacalcet, secondary to emesis or effects on food consumption and body weight gain, and/or observed previously in adult animals.

PS 780 QUANTIFIED TRACKING AND MONITORING OF DIAZEPAM TREATED SOCIALLY HOUSED CYNOMOLGUS MONKEYS.

C. Rose[1], J. E. van der Harst[2], R. C. de Heer[2], B. M. Spruijt[2] and S. H. Korte[1]. [1]*Covance Laboratories GmbH, Muenster, Germany and* [2]*Delta Phenomics BV, Utrecht, Netherlands.* Sponsor: G. Weinbauer.

The objective of this study was to assess effects of diazepam using the automated analysis of digitized video images (EthoVision XT, Noldus IT, The Netherlands) of group housed cynomolgus monkeys (Macaca fascicularis). Males (n = 3) and females (n = 3) were injected with Diazepam (1.0 mg/kg) and behavioural parameters related to activity, locomotion and spatial movements were analysed. General parameters such as distance moved and velocity did not reveal the known sedative effects of Diazepam. However, inspection of the automatically generated track images indicated that diazepam treated animals had a more a meandering walking pattern suggesting that Diazepam induced a loss of balance which was regained by corrective movements. Therefore, parameters revealing specific aspects of the movement pattern such as velocity profiles and turn angles have been analyzed. Most prominent effects are represented by changes in the shape of the tracks, shift of the velocity profiles and increases in turn angles. An increase in the mean velocity can be observed in males (+51.3%) and females (+126.5%). In males loss of balance is evidenced by an increase in the circular deviation of the mean turn angle (+18.3%) and a decrease in the duration of movement along a straight line (-34,8%). Females were hardly active during the control situation, which confounded the comparison of control situation versus Diazepam treatment. This study demonstrated that parameters such as distance moved and velocity may be insufficient to distinguish drug effects. The detection of the loss of balance and subsequent corrective movements urged for subsequent adjustment of the analysis protocols. This study demonstrates the indispensable value of automated computerized analysis of video tracking for quantifying differences for different dose levels in pre-clinical safety assessment. Expert-knowledge on the tracking and analysis methods are pivotal for data-interpretation and an optimal use of the techniques used here.

PS 781 28-DAY TOXICITY STUDY WITH THE NON-TRADITIONAL VEHICLE SOLUTOL™ HS15 AND POLYETHYLENE GLYCOL 400 IN FEMALE BEAGLE DOGS.

D. Kemp, J. Hailey, H. Jordan, R. Brown and D. Bailey. *GlaxoSmithKline, Research Triangle Park, NC.*

Conventional vehicles do not always provide adequate systemic exposure of the test article formulation to assess toxicity, in these cases we will consider non-traditional vehicles. The lack of toxicology information with these vehicles has presented a challenge. This study determined the impact of various formulations of Solutol™ HS15 (Solutol)/polyethylene glycol (PEG) 400 on Beagle dogs to determine the tolerability and toxicity following co-administration in a 28-day, oral, repeat-dose study in female beagle dogs. Five groups of dogs (3/group) were given either reverse osmosis-treated water, 10% Solutol/90% PEG 400 (2 mL/kg/day), 30% Solutol/70% PEG 400 (2 mL/kg/day), 10% Solutol/90% PEG 400 (5 mL/kg/day), or 30% Solutol/70% PEG 400 (5 mL/kg/day). All dogs tolerated the administration of 10% Solutol/90% PEG 400 or 30% Solutol/70% PEG 400 at a dose volume of 2 mL/kg/day. Loose/watery feces and minimal mucus-cell hyperplasia of the ileum was present in all groups; however, a higher incidence of loose/watery feces was consistently present in animals given the dose volume of 5 mL/kg/day. Total bilirubin was minimally increased at all doses. An increase in red

blood cell mass and decrease in urine volume in animals given 30% Solutol/70% PEG 400 at a dose volume of 5 mL/kg/day was likely due to dehydration and hemoconcetration associated with the loose/watery feces. Neither 10% Solutol/90% PEG 400 or 30% Solutol/70% PEG 400 at either dose volume produced overt toxicity after 28 days of dosing, however using the lower dose volume of 2 mL/kg/day minimized the incidence of loose/watery feces and potential dehydration.

PS 782 TOXICITY OF MDL72, 527 ADMINISTERED ORALLY TO RATS AND DOGS.

S. D. Grimes[1], R. W. Watkins[1], J. F. Mann[1], H. S. Basu[2], I. M. Kapetanovic[3] and C. D. Hebert[1]. [1]*Southern Research Institute, Birmingham, AL,* [2]*Colby Pharmaceutical Company, Menlo Park, CA and* [3]*National Cancer Institute, Bethesda, MD.*

MDL72,527 is a polyamine oxidase inhibitor that is being developed as a potential chemotherapeutic agent for prostate cancer. The toxicity of MDL72,527 was evaluated in Sprague Dawley rats and beagle dogs. Rats were given MDL72,527 via gavage at doses of 0, 25, 75, 250, or 1000 mg/kg/day daily for up to 14 days or at doses of 0, 25, 50, 100, or 200 mg/kg/day MDL72,527 once every 14 days for 71 days. Initially, dogs were to be given daily doses of MDL72,527 via capsules at doses of 0, 50, 200, or 1000 mg/kg/day for 14 consecutive days. However, because of intolerance to MDL72,527 at higher doses, dogs in the 200 and 1000 mg/kg/day dose groups were dosed for only 7 and 5 days, respectively. Dogs in the 50 mg/kg/day dose group received this dose on Days 1-7, with an increase to 100 mg/kg/day on Days 8-14. Adverse effects associated with giving MDL72,527 daily to rats and dogs included unscheduled mortality, clinical signs of toxicity, decreases in body weight and food consumption, clinical pathology abnormalities, and extensive microscopic lesions. Excluding a thin carcass in one dog, no test article-related gross lesions were observed in rats and dogs given MDL72,527. Microscopic lesions in dogs and rats receiving MDL72,527 daily were multisystemic, consisting of cytoplasmic vacuolization of numerous tissues, particularly the vasculature. Animals given MDL72,527 once every 14 days had minimal test article-related clinical signs, clinical pathology abnormalities, and microscopic lesions (apoptosis). Extreme toxicity was associated with daily oral administration of MDL72,527. Administration of the drug every 14 days for 71 days appeared to mitigate adverse effects of the drug at doses where the drug is active in preventing cancer progression in a mouse spontaneous prostate cancer model. A no-observed-adverse effect level was not established for rats or dogs on the dosing schedules used in these studies.
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PS 783 SAFETY ASSESSMENT OF PHOSPHO-IBUPROFEN AS A POTENTIAL CANCER CHEMOPREVENTIVE AGENT.

R. W. Watkins[1], J. F. Mann[1], S. D. Grimes[1], R. Fulton[2], C. V. Rao[3], I. M. Kapetanovic[4] and C. D. Hebert[1]. [1]*Southern Research Institute, Birmingham, AL,* [2]*FVClinPath Consulting, Birmingham, AL,* [3]*University of Oklahoma Health Sciences Center, Oklahoma City, OK and* [4]*National Cancer Institute, Bethesda, MD.*

Phospho-ibuprofen (pIB) is being evaluated for use as a cancer chemopreventive agent, and is believed to have lower toxicity and greater chemopreventive potency than ibuprofen itself. To assess the toxicity and pharmacokinetic behavior of pIB in rats, two studies were performed in which Sprague Dawley rats received daily oral gavage doses of pIB at 0, 25, 50, 150, or 450 mg/kg/day for 14 days or 0, 20, 100, or 500 mg/kg/day for 28 days. One rat died in the high dose group of each study. Administration of 150 or 450 mg/kg/day produced minimal clinical signs and minimal changes in neutrophil, globulin, and albumin values. In the 14-day study, a no-observed-adverse-effect level (NOAEL) dose was not identified. pIB metabolites in plasma included R- and S-ibuprofen, hydroxyphospho-ibuprofen, 1- and 2-hydroxyibuprofen, and ibuprofen glucuronide. In the 28-day study, there were no clinical signs of toxicity attributed to pIB administration. Rats in the two highest dose groups had minimal (≤10%) deficits in body weight gain and food consumption compared to controls. Clinical pathology alterations in leukocytes (high leukocytes, neutrophils, and monocytes), serum protein (low total protein, albumin, and albumin/globulin) and/or high fibrinogen values observed for rats in the two highest dose groups were consistent with inflammatory and necrotic effects observed in various tissues of animals in this study, as well as with the known toxic effects of ibuprofen. Microscopic lesions consistent with ibuprofen toxicity were observed in the glandular stomach, forestomach, large intestine, kidney, and liver of pIB-treated rats. Based on microscopic changes observed in the gastrointestinal tract and kidney at the 20 mg/kg/day dose level, a NOAEL dose was not identified for rats dosed with pIB daily for 28 days.
Funded wholly with federal funds from National Cancer Institute Contract HHSN26120043307C

PS 784 EARLY SAFETY ASSESSMENT CONSIDERATIONS AND STRATEGIES IN DRUG DISCOVERY.

S. Mohr[1], A. J. Olaharski[2], F. Crameri[1], T. Singer[1] and T. Weiser[1]. [1]*Nonclinical Safety, F. Hoffmann-La Roche, Basel, Switzerland and* [2]*Nonclinical Safety, F. Hoffmann-La Roche, Nutley, NJ.*

The likelihood that a new chemical entity progresses through the pre-clinical stages to market is very low. While there are many factors that contribute to the failure of developing novel pharmaceuticals, one of the largest has historically been due to safety. Because drug failures are tremendously expensive, both in terms of time and money, a number of strategies have been developed to reduce attrition. Traditionally, non clinical safety representation within Roche began once a clinical lead had been selected and safety studies to support entry into human were planned. This process resulted in a large number of unexpected toxicities observed during the first repeat dose safety studies and a high attrition rate. Approximately 5 years ago, an early safety representative (ESR) group was formed to provide active safety support to project teams during all the early drug discovery phases (from target assessment to clinical lead selection). One area of focus has been in the form of a thorough early safety target assessment which provides a non-clinical safety (NCS) functional opinion on the potential safety liabilities, strategies to adress these liabilities and an assessment of whether the putative target should be pursued. Additionally, a number of in silico, in vitro and in vivo mechanistic/ranking approaches are specifically applied to better select drug candidates. This philosophy is conceptually very simple; reduce compound attrition through proactive and collaborative safety representation at earlier phases of drug discovery, identifying potential target-related safety liabilities during target assessment and the generation of more predictive safety data during the lead optimization phase prior to clinical lead selection. Specific examples of how this approach has been applied within Roche will be provided. Using this approach, Roche was able to markedly reduce the safety-related attrition of the selected clinical leads .

PS 785 ASSESSMENT OF HEMATOLOGICAL TOXICITY ASSOCIATED WITH COMBINING CLASSICAL CHEMOTHERAPEUTIC AND TARGETED CANCER DRUGS.

S. Khoh-Reiter, D. Lee and B. Jessen. *Pfizer Inc., San Diego, CA.*

In the past standard treatment for cancer was chemotherapy. While effective in combating cancer, there are often undesirable side-effects with hematological adverse effects being the most common. In an attempt to decrease the toxicity profile of the treatments, rationally designed drugs or targeted therapies are being developed, such as kinase inhibitors. Another approach undertaken by oncologists is combining more than one drug to treat the cancers, aptly called combination therapy. The purpose of this study is to propose a predictive method for determining the adverse effects of combining classical chemotherapy and a targeted therapy on bone marrow cells. The ability to know whether the effects of combining drugs will produce a synergistic, additive or similar toxicity profile as the monotherapies will provide invaluable data for patient treatment plans. Five compounds, three of which are kinase inhibitors, Sorafenib, Imatinib and a CHK-1 compound; a taxane, Paclitaxel; and a classical nucleoside analog, Gemcitabine were tested using differentiated in vitro human bone marrow mononuclear cells, which can give rise to various hematopoietic lineages. Dose-dependent decrease in cell viability in the progenitor population was determined using cellular ATP as the biomarker. A synergistic response was obtained through the combination of Gemcitabine and the CHK-1 compound. In the Sorafenib-Paclitaxel combination, an additive decrease in the cell viability was observed compared to the treatments of the single therapies alone. To a much lesser extend, Gemcitabine combined with Sorafenib or Imatinib did not produce an additive response. In summary, these results demonstrate the feasibility of using human bone marrow mononuclear cells to test different combination therapies for oncology and can subsequently be used as a research tool for predicting neutropenia for various single or combination therapies for either developing compounds or established therapies.

PS 786 HUMAN NON-RELEVANCE OF MOTILIN AGONIST INDUCED ANAPHYLACTOID REACTIONS IN BEAGLE DOGS.

P. Chowdhury and C. Powell. *Safety Assessment, GlaxoSmithKline, Ware, United Kingdom.*

Background: Toxicities in animals must be assessed for human relevance. GSK962040 & GSK1322888 (MW 461 & 434, respectively) are motilin receptor agonists, in development as gastric prokinetics. Methods: Single oral doses of GSK962040 were given to beagle dogs or Göttingen minipigs. Daily oral doses of

GSK962040 were given to dogs (up to 7d) minipigs (up to 1 mo) rats & mice (up to 3 mo) & rabbits (up to 11 wk). Doses were limited by tolerability & were up to 400-fold the max clinical dose (mg/kg basis). Daily oral doses of GSK1322888 were given to dogs (up to 1wk), minipigs (up to 1 mo) & rats (up to 1 mo) also limited by tolerability, which was lower than for GSK962040. Toxicokinetics, haematology, clinical chemistry, urinalysis, ECG, histopathology & plasma histamine were examined. Oral Phase I clinical studies in 92 subjects, given 1-14 daily doses of up to 150 mg GSK962040, examined pharmacokinetics, haematology, clinical chemistry, urinalysis & ECG. Results: 16/16 dogs given GSK962040 or GSK1322888 showed emesis; swollen &/or pink muzzle, eye lids, ears &/or face, gums; reddening of neck, dorsal abdomen & groin, from 15 mins up to 2 hrs post admin. Reactions were independent of dose or duration of dosing. Peak responses correlated with a ≥4.4-fold increased plasma histamine (≥100 nmol/L). Only emesis was noted in the minipig & no reactions occurred in rats, mice, rabbits or humans. GSK962040 exposure (AUC) in dogs at the lowest dose causing anaphylactoid reaction (10 mg/kg/day), was lower than minipig (0.7-fold) rabbit (0.6 -fold), mouse (0.1 fold) or rat (0.7-fold). Exposure in the dog at the lowest effect dose was 0.3-fold that in humans at the highest dose given (3 mg/kg). Conclusion: Dogs (but not 5 other species, including humans) rapidly developed anaphylactoid reactions after exposure to 2 motilin agonists. For these 2 compounds, the minipig was a more appropriate human model than the dog. Plasma histamine was a good biomarker of such anaphylactoid reactions, which are a consequence of mast cell degranulation.

PS 787 AN IMPROVED ORAL TOXICITY PROFILE OF A NOVEL BOTULINUM TOXIN TYPE A FORMULATION.

H. F. Stone, T. D. Thach, L. Garcia and C. L. Ruegg. *Revance Therapeutics, Newark, CA.*

Botulinum neurotoxins produced by anaerobic bacteria of the genus *Clostridium* are the most toxic proteins known. Botulinum neurotoxin serotype A (BoNTA) is initially released from the bacterial cell as a stable 900-kDa complex comprised of the neurotoxin along with several additional bacterial proteins. The 900-kDa BoNTA complex is one of the forms of the toxin employed as a therapeutic agent for the treatment of various neuromuscular disorders and other cosmetic indications. A novel BoNTA formulation, RT001 (purified 150-kDa BoNTA lacking additional complex proteins in a proprietary formulation) is currently studied as an investigational new drug for topical treatment of aesthetic and therapeutic indications. In this study, the oral toxicity of RT001 was compared to the 900-kDa BoNTA complex. When exposed to simulated gastric fluid (USP), RT001 bioactivity was undetectable within 1 minute whereas the half-life of the 900-kDa BoNTA complex was approximately 20 minutes, indicating that the additional components of the 900-kDa BoNTA complex protect the toxin from the harsh conditions of the gastrointestinal track (acidic pH and gastric proteases). Consistent with this *in vitro* data, the oral toxicity of RT001 in rats was significantly reduced as compared to the 900-kDa BoNTA complex. The improved oral toxicity profile of RT001 provides a scientific basis for potential therapeutic applications uniquely suited for this new form of toxin. (All animal experimentation was carried out in accordance with the Society's criteria and use of animals in research).

PS 788 NONCLINICAL TOXICOLOGY OF BIIB022, AN ANTI-IGF-1R MONOCLONAL ANTIBODY.

K. Stickland[1], E. Polack[1], J. Galluppi[1], J. Morrow[1], J. Clarke[1], S. Chuang[2], S. Tichenor[2], L. Huard[3] and S. Rose[2]. [1]*Biogen Idec, Cambridge, MA,* [2]*Charles River Laboratories, Reno, NV and* [3]*Charles River Laboratories, Montreal, QC, Canada.*

BIIB022, an antibody antagonist to the Insulin-Like Growth Factor 1 Receptor (IGF-1R), was designed to inhibit pro-survival and tumor proliferation pathways by down regulation of the IGF-1R receptor and by blocking IGF-1 and IGF-2 signaling interaction with the IGF-1R receptor. The Fc portion of BIIB022 is non-glycosylated IgG4; due to the lack of glycosylation, the Fc portion of the antibody is not active in terms of antibody-dependent cell mediated toxicity; This combination eliminates effector function (e.g., ADCC) with the goal of achieving superior safety. BIIB022 is currently in Phase 1/2 trials in solid tumors.
A number of toxicity studies were conducted to support clinical development of BIIB022: A tissue cross reactivity study on normal human and non-human primate tissues; hemolytic potential and blood compatibility in human blood; and repeat dose toxicity studies in monkeys (4- and 26-week toxicology studies with recovery periods). The tissue cross reactivity study indicated similar cross reactivity of BIIB022 in human and monkey tissues. The in vitro hemolytic potential and blood compatibility study indicated that BIIB022 is compatible with IV administration in humans. In repeat dose studies, BIIB022 was administered (IV) once weekly, to match the most aggressive scheduling that may be tested clinically. No target organs of toxicity were identified in 4- or 26-week studies at doses up to 50 mg/kg. Additionally, no BIIB022-related changes in clinical pathology parameters were noted. There was an apparent trend for decreases in primary humoral response to Tetanus Toxoid antigen during the recovery phase of the 26-week study, however, these findings were not considered to be adverse. The NOAEL in the 4- and 26-week studies was considered to be 50 mg/kg. The non-clinical safety data of BIIB022 in monkeys support safe use conditions in ongoing clinical trials.

PS 789 PPD10558: AN ORALLY EFFECTIVE STATIN WITHOUT MYOTOXIC EFFECTS.

R. A. Lopez[1], D. Prohaska[1], G. McIntyre[1] and M. Griggs[2]. [1]*Furiex Pharmaceuticals, Inc., Morrisville, NC and* [2]*MPI Research, Mattawan, MI.*

Myotoxicity remains a main safety concern with HMG-CoA reductase inhibitors (statins). PPD10558 (558) is a statin in clinical development with very low systemic exposure. To determine if 558 has an optimal safety profile, the toxicity, pharmacokinetics, and select tissue distribution of 558 were compared to those of atorvastatin (ATV) and pravastatin (PRV) in rats at a dose of 350 mpk PO for up to 14 days. Drug levels were determined in plasma and in tissue samples via LCMS.
In a 14 day study comparing 558 and ATV, all rats given 558 survived until termination and the only 558-related effect was mild elevations of AST and ALT. However, 7 of the 10 males given 350 mpk PO ATV were found dead beginning on Day 12. The cause of death was skeletal muscle degeneration/necrosis. In the ATV animals, significant decreases in body weight (BW) and food consumption (FC) were observed, as well as moderate to marked elevations of AST, ALT, LDH, creatine kinase, and urea nitrogen. ATV-related microscopic observations were present in skeletal muscle and kidneys. The absolute bioavailability of 558 and ATV were 0.2% and 3.0%, respectively.
In a 10 day study comparing 558, PRV and ATV, all animals survived to termination. ATV-treated animals showed adverse decreases in BW and FC. ATV dosed animals exhibited much greater elevations of AST and ALT than those treated with 558 or PRV, which only had non-adverse mild elevations. The levels of ATV and metabolites in muscle, liver, kidney and plasma were considerably higher than levels of PRV, 558 and PPD11901 (901: 558 metabolite). Notably, in muscle, ATV and PRV concentrations were 39x and 3x higher than 558, respectively. Although no muscle degeneration was observed in any group, muscle concentrations of all statins tested were higher than the IC50 needed to inhibit HMGCoA reductase in vitro.
The findings from both studies suggest that accumulation of ATV and metabolites in muscle after repeat dosing in rats causes myotoxicty, while the lack of findings in 558-treated animals are possibly due to the very low levels of 558 and 901 observed in the muscle.

PS 790 COMPREHENSIVE INVESTIGATION OF PROPYLENE GYLCOL, POLYSORBATE 80, AND HYDROXYPROPYL-BETA-CYCLODEXTRIN FOR USE IN GENERAL, REPRODUCTIVE, AND DEVELOPMENTAL TOXICOLOGY STUDIES.

E. A. Thackaberry[1], S. Kopytek[2], P. Sherratt[3], K. Trouba[4], B. McIntyre[5], K. A. Treinen[2] and B. P. Enright[6]. [1]*Safety Assessment, Genentech, South San Francisco, CA,* [2]*Safety Assessment, Merck Research Laboratories, Summit, NJ,* [3]*Toxicology, Celgene Corporation, Summit, NJ,* [4]*Drug Safety Evaluation, Bristol-Myers Squibb, Mt. Vernon, IN,* [5]*National Toxicology Program, National Institute of Environmental Health Science, Research Triangle Park, NC and* [6]*Preclinical Safety, Abbott Laboratories, Abbott Park, IL.*

Studies were conducted to assess the safety and tolerability of Polysorbate 80 (PS80), Propylene Gylcol (PG) and Hydroxypropyl-Beta-Cyclodextrin (HPβCD) in nonclinical general, reproductive, and developmental toxicology studies. In the general toxicology studies, 10 mg/kg PS80, 1000 mg/kg PG, 500 mg/kg HPβCD or 1000 mg/kg HPβCD were administered by oral gavage to mice, rats, dogs, and cynomolgus monkeys for approximately 90 days. In the fertility study, MC, PS80, PG, and HPβCD were administered at the same doses prior to/during mating, and on gestation Day (GD) 0 to 7, followed by an assessment of embryonic development on GD 14. In the teratology studies, pregnant female rats and rabbits were administered the same doses of MC, PS80, PG, and HPβCD during the period of organogenesis, followed by an assessment of fetal external, visceral and skeletal development. The suitability of formulations containing up to 20 mg/kg MC, 10 mg/kg PS80, and 1000 mg/kg PG for use in nonclinical safety studies was confirmed by a lack of effects on all parameters examined. However, formulations containing HPβCD produced elevated transaminase levels in rats and mice, fecal changes in dogs and monkeys, and maternal toxicity in the rabbit teratology study, which included stool findings, decreased food consumption and body weight gain, and evidence of abortion. Based on these data, PS80 and PG are considered to be

practical alternatives to MC in nonclinical toxicology studies. However, formulations containing HPβCD should be used with caution due to elevations in rodent transaminase levels and maternal toxicity in pregnant rabbits.

PS 791 NONCLINICAL DEVELOPMENT OF BMN 110, AN ENZYME REPLACEMENT THERAPY FOR MUCOPOLYSACCHARIDOSIS TYPE IVA (MPS IVA).

F. Lorget, D. Kennedy, Y. Qi, L. Argento Martell, A. Van Tuyl, M. Dvorak-Ewell, E. Paysinger, C. O'Neill and L. Tsuruda. *BioMarin Pharmaceuticals Inc., Novato, CA.*

BMN 110 is being developed as an enzyme replacement therapy for the treatment of MPS IVA, a lysosomal storage disorder caused by a deficiency of the enzyme N-acetylgalactosamine-6 sulfatase that results in accumulation of the glycosaminoglycan keratan sulfate (KS) leading to skeletal dysplasia, main clinical manifestation of the disease. The prevalence of MPS IVA is estimated at 1 in 200,000 world wide. After IV administration, BMN 110 is taken up into the cell and localized to the lysosomes via Mannose 6 Phosphate targeted delivery. Enzyme uptake into the lysosome is expected to increase KS catabolism in affected tissues, thus reducing the progressive accumulation of KS. In absence of a correlative animal model of the disease, BMN 110 pharmacological activity was demonstrated in human MPS IVA primary chondrocytes. Treatment of these cells with BMN 110 (10 nM) resulted in KS clearance and normalization of the expression profile for some chondrogenic genes such as the master transcription factor Sox9. BMN 110 toxicity was evaluated in two repeat dose toxicity studies, one in the rat and one in the monkey over 26 and 52 weeks, respectively. The animals received weekly BMN 110 administration at dose levels up to 20 mg/kg via IV (IV bolus for the rat and IV 4 hr infusion for the monkey). To support pediatric use of BMN 110, juvenile monkeys were utilized. Toxicological effects of BMN 110 were limited to those expected following the administration of a heterologous protein: 1) species-specific anaphylactoid-type reactions in the rat but not in the monkey and, 2) formation of total and neutralizing (binding) anti-BMN 110 antibodies. BMN 110 administration was well tolerated in rats pretreated with diphenhydramine and in monkeys after weekly BMN 110 administrations at dose levels up to 20 mg/kg/week over 26 and 52 weeks, respectively. No target organ toxicity was observed. No exaggerated pharmacological effects on bone and cartilage growth or KS turnover were observed in these normal animals.

PS 792 GASTROINTESTINAL TOXICITY IN RATS FOLLOWING ORAL ADMINISTRATION OF AURORA KINASE INHIBITORS.

M. J. Anderton[1], H. Mellor[1], J. Barnes[1], T. Child[1], N. Barrass[1], P. Cotton[1], S. Green[2], J. Foster[1] and R. Roberts[1]. *[1]Safety Assessment UK, AstraZeneca, Cheshire, United Kingdom and [2]Cancer Research Department, AstraZeneca, Cheshire, United Kingdom.*

Aurora kinases are important regulators of mitosis and cytokinesis that are aberrantly expressed in a wide range of solid and hematological malignancies. There has been a drive to develop aurora kinase inhibitors for the treatment of human malignancies, particularly acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Both intravenous and oral routes of administration are being explored in clinical trials.

Previously following i.v. administration of aurora kinase inhibitors we have observed bone marrow hypocellularity and neutropenia as the dose-limiting toxicity in pre-clinical studies. Reports from clinical trials have also indicated neutropenia as dose-limiting for this target. The reduction in the numbers of cells from myeloid and lymphoid lineages is considered desirable for indications targeting hematological malignancies.

In the current study, the pre-clinical safety of aurora kinase inhibitors from three different chemistry scaffolds was explored following oral administration to rats. Ten-week-old female Han Wistar rats received test compounds for 4-5 days. Necropsy was performed 24h after the final dose and tissues were processed for histopathology. Consistent with previous findings with i.v. aurora kinase inhibitors, all three compounds induced bone marrow hypocellularity and severe neutropenia. At doses that induced a comparable level of neutopenia to the i.v. compounds, the oral inhibitors were associated with severe mucosal atrophy in the jejunum, duodenum, ileum and caecum. The atrophy was characterised by villous blunting and fusion with attenuation of the epithelium and marked cellular pleomorphism and karyocytomegaly. These findings suggest that administration of aurora kinase inhibitors via the oral route in the clinic may be complicated by associated gastrointestinal toxicity.

PS 793 HSP90 INHIBITORS REDUCE ELECTRORETINOGRAM RESPONSES IN DOGS.

C. Somps[1], C. Liu[1], N. Poy[3], J. Marcek[1] and V. Torti[2]. *[1]Drug Safety R&D, Pfizer, Inc., Groton, CT, [2]Drug Safety R&D, Pfizer, Inc., La Jolla, CA and [3]World Wide Comparative Medicine, Pfizer, Inc., Groton, CT.*

Inhibitors of the molecular chaperone heat shock protein-90 (HSP90) are being developed to treat various cancers. Two HSP90 inhibitors, a prodrug PF-04929113 and a structurally distinct active inhibitor, have been associated with alterations in visual responses in dogs noted on ophthalmologic examination following 2-3 weeks of daily oral dosing, and associated with irreversible photoreceptor loss on histopathology examination following 2-4 weeks of daily dosing. In the present study, electroretinography (ERG) was used to determine if functional changes in retinal response could be detected in dogs dosed daily with either compound, and whether changes could be detected early enough to be used as a leading biomarker of retinal toxicity. Under general anesthesia, ERGs were acquired from 4 dogs following 4 and 11 days of dosing and compared to ERGs obtained from the same 4 dogs pre-study. Results showed reductions in ERG waveform amplitudes as early as Day 4, which progressed in severity on Day 11. In the dark-adapted retina both a-wave and b-wave amplitudes (rod and cone response) were significantly decreased on Day 4 (50-60%, $p < 0.001$) and Day 11 (80-90%, $p < 0.001$) for both compounds. Similarly, in the light-adapted retina the b-wave amplitude (cone response only) was significantly reduced for one of the compounds on Day 4 (30-40%, $p < 0.01$), and for both compounds on Day 11 (60-70%, $p < 0.001$). In the same animals, ocular pharmacokinetic analysis indicated these compounds (or their active metabolites) were distributed to the retina at concentrations 4-11 fold those detected in plasma. Given the similarity of visual effects observed for the 2 structurally distinct HSP90 inhibitors reported here, our results suggest that HSP90 is critical to normal visual function, and that prolonged inhibition of HSP90's molecular chaperone function is associated with functional deficits in vision which may progress to irreversible retinal damage.

PS 794 SUBCHRONIC ORAL TOXICITY EVALUATION OF CP31398, A P53 STABILIZER, IN RATS.

D. L. McCormick[1], W. D. Johnson[1], M. Muzzio[1], C. J. Detrisac[2], L. Kopelovich[3] and I. M. Kapetanovic[3]. *[1]IIT Research Institute, Chicago, IL, [2]Pathology Associates, Chicago, IL and [3]National Cancer Institute, Bethesda, MD.*

Stabilization of p53 activity may provide a useful approach to cancer prevention and therapy. CP31398 is a styrylquinazoline that stabilizes the DNA binding conformation of p53, thereby maintaining the activity of p53 as a transcription factor and tumor suppressor. A subchronic toxicity study was performed in which groups of 15 CD rats/sex received daily gavage exposure to CP31398 at 0, 40, 80, or 160 mg/kg/day (0, 240, 480, or 960 mg/m^2/day) for 28 days. Ten rats/sex/group were scheduled for necropsy on Day 29; remaining rats were necropsied after a 14-day recovery period. Eleven rats (7 males, 4 females) from the high dose group died during the dosing period; no deaths were seen at lower doses. At necropsy, extensive gross pathology was seen in the high and mid dose groups; changes were more severe at the high dose. No gross pathology was seen in the low dose group. Histopathologic evaluation of tissues identified treatment-related alterations in the liver, kidney, heart, bone marrow, lung, adrenals, spleen, thymus, skeletal muscle, and ovary in the high dose group. In the mid dose group, microscopic changes were seen in the liver, kidney, heart, lung, adrenals, and spleen. Histopathologic changes in the liver, heart, lung, and adrenals remained in the high and mid dose groups after the recovery period. Microscopic changes in the low dose group were limited to apparently reversible alterations in the lungs, spleen, and heart. Clinical pathology data provided evidence of hepatic toxicity, renal toxicity, anemia, inflammatory reactions, and coagulopathies in the high and mid dose groups. The liver appears to be the primary site of limiting toxicity for CP31398 in rats. Because histopathologic changes were seen in all dose groups, a No Observed Adverse Effect Level (NOAEL) for CP31398 could not be established. The Maximum Tolerated Dose (MTD) for subchronic oral administration of CP31398 in rats is 80 mg/kg/day (480 mg/m^2/day). (NCI-N01-CN-43304)

PS 795 SUBCHRONIC ORAL TOXICITY EVALUATION OF CP31398, A P53 STABILIZER, IN DOGS.

W. D. Johnson[1], M. Muzzio[1], C. J. Detrisac[2], L. Kopelovich[3], I. M. Kapetanovic[3] and D. L. McCormick[1]. *[1]IIT Research Institute, Chicago, IL, [2]Pathology Associates, Chicago, IL and [3]National Cancer Institute, Bethesda, MD.*

CP31398 (N'-[2-[2-(4-methoxyphenyl)ethenyl]-4-quinazolinyl]-N,N-dimethyl-1,3-propanediamine dihydrochloride) is a small molecule that can (a) restore p53 function in cells expressing mutant p53; (b) increase the activity of wild-type p53 in

cells; (c) induce cell cycle arrest and apoptosis in cancer cells in vitro; and (d) inhibit the in vivo growth of human tumors in xenograft models. To evaluate the subchronic oral toxicity of CP31398 in a non-rodent model, groups of six beagle dogs/sex received daily gavage exposure to CP31398 at doses of 0, 10, 20, or 40 mg/kg/day (0, 200, 400, or 800 mg/m^2/day) for 28 days, followed by a 14-day recovery period. Two female dogs in the high dose group died prior to the Day 29 necropsy; both early deaths demonstrated hepatic necrosis without evidence of thrombosis. Although gross pathology was unremarkable, histopathologic findings in dogs surviving until Day 29 included perivascular lymphocytic infiltration in the brain and spinal cord, gliosis in the brain, alveolar histiocytosis in the lung, and deposition of basophilic material in hepatocytes and in splenic macrophages. Microscopic changes in the CNS, lung, and liver were still present in dogs necropsied on Day 43. During the in-life period, CP31398 induced sporadic emesis in both sexes in the mid and high dose groups, and significant reductions in body weight gain in all dose groups. CP31398 induced anemia in both sexes in all dose groups; anemia was not fully reversed at the end of the recovery period. In consideration of the effects of CP31398 on body weight and red cell parameters in all dose groups, and on the basis of microscopic changes identified in the CNS, lung, and liver in these groups, a No Observed Adverse Effect Level (NOAEL) could not be established for CP31398 in this study. The Maximum Tolerated Dose (MTD) for subchronic oral administration of CP31398 in dogs is 20 mg/kg/day (400 mg/m^2/day). (NCI-N01-CN-43304)

PS 796 SUBCUTANEOUS ADMINISTRATION STUDY TO DETERMINE THE INFLUENCE OF AN ANTI-MOUSE IL-12/IL-23P40 ANTIBODY ON PHOTOCARCINOGENESIS IN HAIRLESS MICE.

W. M. Bracken[1], D. Learn[2], G. Blaich[1] and G. M. Veldman[3]. *[1]Global Preclinical Safety, Abbott, Abbott Park, IL, [2]Photobiology, CRL, Horsham, PA and [3]Biologics Generation, Abbott Bioresearch Center, Worcester, MA.*

An anti-mouse IL-12/IL-23p40 surrogate of ABT-874 (8D12) was administered by the subcutaneous route and evaluated for the potential to influence the development or growth of skin tumors in hairless mice exposed to simulated solar ultraviolet radiation (UVR). Albino hairless mice (Crl:SKH1-hr), 24 per sex per group, were assigned to the study. Treatment groups included: 8D12 plus 600 RBU solar simulated ultraviolet radiation; 8D12 without UVR exposure; vehicle control with UVR exposure at either 600 or 1200 RBU; and vehicle control without UVR exposure. 8D12 was administered once weekly at 31 mg/kg for 40 weeks. UVR exposure occurred 5 days per week. After administration and UVR exposure was completed, the mice were held an additional period to allow all UVR exposed groups to achieve at least median latent period of ≥2 mm tumors. Serum concentrations of 8D12 were maintained throughout the study period.The skin tumor response in the group administered 8D12 and exposed to UVR was similar to the skin tumor response in the group of mice administered the vehicle control and exposed to the same UVR dose. Subcutaneous administration of 8D12 without UVR exposure did not result in any skin tumor response or other adverse skin reactions over the course of the study. The skin reactions in the groups exposed to UVR were attributed to UVR exposure alone, not to any effect of subcutaneous 8D12 administration. No mortality, clinical observations, necropsy observations or body weight effects were considered related to vehicle control or 8D12 administration, without or with UVR exposure. Based on the results of this study, repeated (once) weekly subcutaneous administration of 8D12 at 31 mg/kg with exposure to UVR for five days per week for a total of 40 weeks of administration did not modify photocarcinogenesis in Crl:SKH1-hr albino hairless mice, in comparison with UVR exposure only.

PS 797 ANTIBODY-MEDIATED VASCULITIS: *IN VIVO* INVESTIGATIONS INTO THE MECHANISM OF TOXICITY.

K. Mackay[1], T. Sullivan[1], M. Carioto[1], J. Senn[1], J. Lane[2], J. S. Michaelson[3] and J. C. Clarke[1]. *[1]Pharmacotoxicology, Biogen Idec, Cambridge, MA, [2]Comparative Pathology, Biogen Idec, Cambridge, MA and [3]Immunobiology, Biogen Idec, Cambridge, MA.*

Compound X is a full length humanized agonist monoclonal antibody that is currently in preclinical development. In an initial 28-day, repeat dose IND-enabling study in nonhuman primates (NHP), once weekly administration of compound X was associated with arteritis in several organs at all 3 doses tested. The arteritis affected small, medium caliber arteries/arterioles, had a low, sporadic incidence/distribution, and no clear clinical pathology correlate was identified. There was evidence of the arteritis resolving following an 8-week, treatment free recovery period. A NOAEL was not established, and safe use conditions for first in human (FIH) studies were not defined. To investigate the mechanism of toxicity, additional studies were performed in mice, the species used in pharmacology efficacy studies. These studies permitted the examination of the roles of binding, signaling, and effector function, and utilized adenoviral vector delivery of soluble ligand, administration of several isotypes of ligand-Fc fusion proteins, and administration of several isotypes of the murine homolog of compound X. Arteritis was observed in mice under all treatment conditions, but not in control animals. The arteritis was characterized by minima/mild periarterial infiltrates with minimal evidence of structural changes to vessel walls, and mild/moderate mixed cell arteritis. Both patterns had a low incidence, and were most prevalent in the lung. Based on these data, arteritis appears to be linked linked to pathway irrespective of the mode of agonism, intact Fc function may not be required to induce arteritis, and the mouse is potentially an appropriate preclinical species to further investigate arteritis associated with compound X. On-going studies include investigating if compound X induces arteritis in mice, and the identification of an associated NOAEL and appropriate therapeutic index. Safe use conditions for FIH studies would then be defined in GLP studies in NHP.

PS 798 INHIBITION OF THE ENZYME S-NITROSOGLUTATHIONE (GSNO) REDUCTASE DOES NOT CAUSE MECHANISM-BASED TOXICITY.

D. B. Colagiovanni, X. Sun, J. Qiu, A. Stout, A. Patton, L. Green, J. Richards and G. J. Rosenthal. *Research, N30 Pharmaceuticals, Boulder, CO.*

S-nitrosoglutathione (GSNO) is an endogenous nitrosothiol that serves as both a donor and depot for nitric oxide (NO). The enzyme GSNO reductase (GSNOR), also known as aldehyde dehydrogenase 5 or formaldehyde dehydrogenase, catalyzes the metabolism of GSNO and controls levels of intracellular s-nitrosothiols. Given its central role in controlling NO stores and formaldehyde levels, it is important to understand potential negative consequences of modulating GSNOR. Fortunately, other enzymes are capable of catalyzing the breakdown of formaldehyde, including ADH 1A1 and 2. Based on studies using exogenous NO donors, NO-mediated effects from reduced breakdown could include hypotension, methemoglobinemia and changes in platelet aggregation. We evaluated potential deleterious effects of GSNOR inhibition using small molecule inhibitors, agents being developed for treatment of respiratory diseases. The inhibitory activity of these compounds is in the low nanomolar range. Potency and specificity of the inhibition of GSNOR was assessed by in vitro enzymatic activity measurements using purified recombinant human GSNOR and by comparing compound inhibition of GSNOR with other classes of ADH enzymes. In vitro screening assays and exploratory rodent toxicology studies were conducted with five representative compounds. Assays included assessments of total GSH, GSH synthesis, lipid peroxidation, mitochondrial function and apoptosis. In vivo studies evaluated general toxicity and cardiac function. Results demonstrate that pharmacologic inhibition of GSNOR does not adversely impact "on target" endpoints traditionally associated with increased systemic NO and class effects were not seen. In vitro assays showed mild toxicity only at exceedingly high doses, while there were no target organs of toxicity in the in vivo studies. These findings indicate that inhibiting GSNOR does not lead to toxicity and further evaluation of novel inhibitors of the enzyme is warranted.

PS 799 ORAL TWO WEEK (3X/WEEK) DOSE RANGE FINDING STUDY OF DECITABINE AND TETRAHYDROURIDINE IN CD-1 MICE.

K. Engelke[1], E. Hanan[1], J. Covey[2], Y. Ling[3], K. Chan[3], Y. Saunthararajah[4] and P. Terse[2]. *[1]AVANZA Laboratories, LLC, Gaithersburg, MD, [2]National Cancer Institute, Bethesda, MD, [3]Ohio State University, Columbus, OH and [4]Cleveland Clinic, Cleveland, OH.*

The purpose of this study was to determine the target organ toxicity and reversibility of decitabine (DAC) and tetrahydrouridine (THU; administered 1 hour prior to DAC) in mice treated orally 3 times/week for up to 7 doses followed by a 2-week recovery period. Twelve animals/sex received both THU vehicle and DAC vehicle, or 167 mg/kg THU followed by DAC at 0, 0.4, 2, or 10 mg/kg. Treatment with four or five doses of 167 mg/kg THU/10 mg/kg DAC resulted in moribundity or mortality in all mice. Clinical findings in these animals included hunched and languid appearance, rough haircoat and decreased body weight; decreased leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin, hematocrit, platelet and reticulocyte counts. Treatment with 167 mg/kg THU/2 mg/kg DAC resulted in similar clinical observations and moribundity/mortality in 1 male and 3 females. Gross pathology was generally unaffected in early death animals; histopathology was not performed. All surviving an-

imals dosed with 167 mg/kg THU alone or 167 mg/kg THU/0.4 to 2 mg/kg DAC showed no adverse clinical signs, body weight effects, gross necropsy findings or changes in serum chemistry. At terminal sacrifice (SD 16), decreases in leukocyte counts (total and differential, specifically neutrophils and lymphocytes), erythrocyte counts, hemoglobin and hematocrit values were noted in all THU/DAC treated animals. Following the recovery period, all parameters were normal, suggesting reversibility. Cmax was attained at 30 minutes postdose and both Cmax and AUC values were generally proportional to dose. In conclusion, seven doses (3x/week) of 167 mg/kg THU administered alone or 167 mg/kg THU/0.4 mg/kg DAC were generally well-tolerated in male and female CD-1 mice, with the major target of DAC being the hematologic system.
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PS 800 CONSEQUENCES OF REPEATED COMPLEMENT ACTIVATION AFTER CHRONIC TREATMENT IN CYNOMOLGUS MONKEYS.

L. Shen[1], T. Machemer[1], P. Giclas[2] and S. Henry[1]. [1]*ISIS Pharmaceuticals, Inc., Carlsbad, CA and* [2]*National Jewish Health, Denver, CO.*

Antisense oligonucleotide (ASO)-induced complement activation is a class related effect and has been only found in monkeys and not in other species including human. This is a threshold event and associated with higher doses. The consequences of repeated complement activation after chronic ASO treatment were evaluated in cynomolgus monkeys. Animals were treated once weekly with ISIS 104838 for 9 months at 30 mg/kg/week with either subcutaneous (s.c.) injection or 30-minute intravenous (i.v.) infusion. There were transient increases in complement split products (Bb and C3a) and decrease in C3 on Day 1 after a single treatment, which later returned to the baselines. Repeated complement activation resulted in a sustained decrease in plasma C3 after 3 months of treatment that progressed over the course of the study. The complement C3 levels at termination were about 47% and 56% of the baseline for weekly s.c. and weekly i.v. group, respectively. There were also increases in predose levels of the split products for both groups with slightly higher levels (especially Bb) found in the s.c. group. As the results of chronic complement C3 depletion, there were time-dependent increases in circulating immune complex (CIC-C3d) and possible immune-complex mediated pathologies found in the vasculatures of heart, liver and kidney. The incidence and severity of pathology was correlated with the decrease in C3. IgM deposition on vessel wall and C3 staining on macrophages was also confirmed using immunohistochemistry staining in affected tissues. In summary, we found chronic complement activation with ASO in monkey caused C3 depletion which was correlated with impairment of the complement function. These findings are important for the preclinical monkey toxicology evaluation, but have limited impact to clinical safety assessment due to the absence of complement activation in human

PS 801 SINGLE INTRA-CEREBROVENTRICULAR INFUSION TOXICITY, TOXICOKINETICS, AND DISTRIBUTION STUDY OF TRIPEPTIDYL PEPTIDASE-1 IN CYNOMOLGUS MONKEYS.

B. Vuillemenot[1], L. Tsuruda[1], D. Kennedy[1], D. Musson[1], S. Keve[1], R. Reed[2], R. Boyd[2], M. Butt[3] and C. O'Neill[1]. [1]*BioMarin Pharmaceutical Inc., Novato, CA,* [2]*Northern Biomedical Research, Inc., Muskegon, MI and* [3]*ToxPath Specialists, LLC, Hagerstown, MD.*

Neuronal ceroid lipofuscinosis-2 (NCL2) is a lysosomal storage disease caused by tripeptidyl peptidase-1 (TPP1) deficiency and therefore a candidate for enzyme replacement therapy. The objective of this study was to characterize the toxicity, toxicokinetics (TK), and distribution of TPP1 after a single intracerebroventricular (ICV) infusion to cynomolgus monkeys. Animals received 5, 14, or 20 mg recombinant human TPP1 or artificial cerebrospinal fluid (CSF) vehicle via a lateral ventricular catheter. Plasma and CSF were collected for TK analysis during the 3.6 hour infusion and the 14 day recovery period. Animals at all dose levels were necropsied 14 days post-dose. In the vehicle and 14 mg dose groups, animals were also necropsied 3 and 7 days post-dose for temporal assessment of histopathology. Selected CNS tissues were harvested from the 14 mg dose group for TPP1 analysis to assess the tissue distribution. TPP1 infusion had no effect on clinical observations, ECG, or clinical pathology. CSF TPP1 levels peaked at the end of the infusion and displayed biphasic elimination. At the end of the infusion, TPP1 was present in plasma at between 0.1% and 1% of CSF levels. TPP1 was detected in brain tissues 3 days post-dose at 1- to 6-fold endogenous levels. An increase in CSF leukocytes was observed in animals from all groups, including vehicle controls, 24 hours post-dose. There were no differences in blood leukocytes. No TPP1 antibodies were detected in blood or CSF. There were no gross or microscopic pathological effects on the CNS, heart, liver, lung, or kidney attributable to TPP1. This study indicates that ICV infusion of up to 20 mg TPP1 is well tolerated and not associated with any adverse effects over a 14 day recovery period. In addition, the favorable brain distribution and TK of TPP1 after ICV infusion offer promise for the treatment of NCL2.

PS 802 THE SAFETY OF CBD IN SOCIAL ANXIETY DISORDER PATIENTS.

M. M. Bergamaschi[1, 2], M. Chagas[2], D. Oliveira[2], B. Martinis[3], J. Hallak[2], A. Zuardi[2], J. Crippa[2] and R. Queiroz[1]. [1]*School of Pharmaceutical Science of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil,* [2]*School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil and* [3]*School of Philosophy, Science and Literature of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.*

Introduction: Generalized Social Anxiety Disorder (SAD) is one of the most common anxiety conditions, with lifetime prevalence estimates of 7–12% of the general population. SAD is characterized by excessive and persistent fear and anxiety avoidance of a wide variety of social situations. Cannabidiol (CBD), one major non-psychotomimetic compound from the Cannabis sativa, has shown anxiolytic effects both in animals and in human studies. Objective: This study aimed to compare the physiological effects on healthy control (HC) patients and on treatment-naïve SAD patients who received a single dose of CBD or placebo. Method: Twenty-four never-treated patients with SAD were allocated to receive either CBD (600mg; n=12) or placebo (n=12) in a double-blind, randomized design test. The same number of HC (n=12) performed the simulation public speaking test (SPST) without receiving any medication. The protocol was approved by the local Ethical Committee. The groups were matched according to gender, age, years of education and socioeconomic level. Physiological measures [systolic pressure (SP), diastolic pressure (DP), heart rate (HR), spontaneous fluctuation (SF) in skin conductance and skin conductance level (SCL)] were measured at six different time points during the SPST. The results were submitted to a repeated measures analysis of variance (ANOVA). Results: SP, DP, HR, SCL and SF did not show significant differences among the three groups in the all measures, but the HR, which showed a reduction from the initial to the pretest measures significantly greater ($p<0.001$) for the SAD-PLAC than for HC group. The SAD-CBD group did not differ significantly from the other two groups. Conclusion: CBD has shown to be safe for SAD patients and HC, without any significant changes in the physiological measures.

PS 803 *IN VITRO* MABEL APPROACH FOR NONCLINICAL SAFETY ASSESSMENT OF MEDI-565 (MT111), A NOVEL CEA/CD3-BISPECIFIC SINGLE-CHAIN BITE ANTIBODY.

P. C. Ryan[1], S. A. Hammond[1], S. Ren[1], P. Lutterbuese[2], M. Amann[2], M. D. Oberst[1], K. Mulgrew[1], R. Criste[1], S. Fuhrmann[1], N. Lee[1], R. Gross[1], M. Liang[1], A. Schneider[1], R. Dixit[1], P. A. Baeuerle[2], B. Rattel[2], S. Coats[1], L. Roskos[1], K. Bosslet[1], B. Jallal[1] and L. Richman[1]. [1]*MedImmune, Gaithersburg, MD and* [2]*Micromet, Bethesda, MD.*

MEDI-565 (MT111) is a novel bispecific single-chain antibody of the BiTE® (bispecific T cell engager) class that transiently links carcinoembryonic antigen (CEA; also called CEACAM5, CD66e) on cancer cells with CD3 on T cells. Binding of MEDI-565 to CEA and CD3 results in T-cell-mediated killing of cancer cells expressing CEA. MEDI-565 specifically binds to human and cynomolgus monkey CEA with high affinity but not to any other member of the CEACAM family; rodents do not express CEA. MEDI-565 binds to human CD3, but does not bind to CD3 of cynomolgus monkey or mouse. Consequently, no pharmacologically relevant animal species exists for testing the toxicity of MEDI-565. In an effort to introduce a pharmacologically relevant model, two surrogate antibodies were made, cyS111 and hyS111, with specificity to monkey or mouse CD3, respectively. However, the characteristics of these two antibodies were different from those of MEDI-565 to an extent that it was determined that hyS111 and cyS111 would not have utility in nonclinical toxicity studies. Hence, no in vivo toxicology studies were conducted in a relevant animal model with either MEDI-565 or with the two surrogate antibodies. Rather, MedImmune implemented a strategy which utilized an in vitro approach to assess nonclinical safety and select an appropriate first time in human dose for MEDI-565. A minimal anticipated biological effect level (MABEL)

was determined from results of in vitro human tissue culture studies. Exposure parameters were established in a cynomolgus monkey PK study and used to scale PK parameters to humans following allometric correction. Collectively, results from these studies were used to select an appropriate starting dose for Phase 1 clinical studies of MEDI-565 for the treatment of patients with cancers expressing CEA.

PS 804 HEAT SHOCK PROTEIN 90 INHIBITORS CAN INDUCE VISUAL CHANGES AND RETINAL DEGENERATION INCLUDING IRREVERSIBLE PHOTORECEPTOR LOSS IN DOGS.

V. Torti[1], J. Marcek[3], K. Muravnick[3], S. Aguirre[1], J. Fortner[3], J. Render[3], T. Schmahai[3], S. Yamazaki[2], L. Nguyen[2], Z. Shen[2] and L. Burns-Naas[1]. *[1]DSRD, Pfizer Inc., La Jolla, CA, [2]PDM, Pfizer Inc., La Jolla, CA and [3]DSRD, Pfizer Inc., Groton, CT.*

HSP90 inhibitors are currently being tested in the treatment of various cancers. In some of these clinical trials effects on visual field have been reported. To examine this potentially adverse effect more fully, the ocular effects of two structurally-distinct HSP90 inhibitors, one a prodrug (PF-04929113) the other an active form, were tested in dogs. Male and female dogs were dosed for 1-month (QD) in separate studies with each compound. For dogs treated with the active form HSP90 inhibitor, alterations in visual response including apparent blindness and fixed, dilated pupils were observed clinically after 2-3 weeks of dosing. For dogs treated with either inhibitor retinal degeneration was observed that included overt degeneration of the photoreceptor layer and photoreceptor death. The rod photoreceptor cells appeared to be predominantly affected. Photoreceptor death was irreversible. In dogs administered the active form of HSP90 inhibitor for 1-month and allowed to recover for 1-month, there was up to an approximately 50% reduction in observed remaining photoreceptor nuclei. Affected dogs appeared to recover at least part of their detectable visual function after drug administration was suspended. Pharmacokinetics in the eye indicated that distribution of the active metabolite of PF-04929113 to the retina was present at 5-11x the concentration detected in plasma. The active form HSP90 inhibitor had a similar preferential distribution to the retina (4x) relative to plasma. These data suggest that normal HSP90 functioning may be a critical component of retinal and visual function such that prolonged inhibition of HSP90's molecular chaperone functions can lead to functional deficits in vision, structural damage of the retina, and irreversible photoreceptor death.

PS 805 BIOEQUIVALENCY OF TWO DIFFERENT METHOTREXATE FORMULATIONS IN HEPATIC GENE EXPRESSION OF PXB-MOUSE® WITH HIGHLY HUMANIZED LIVER.

M. Nakashima[1], H. Tachiki[2], T. Shimada[3], D. Hynes[4] and S. Nagatsuka[4]. *[1]Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Nagasaki, Japan, [2]Research & Development Division, Towa Pharmaceutical Co., Ltd., Kadoma, Osaka, Japan, [3]PhoenixBio Co., Higashi-Hiroshima, Hiroshima, Japan and [4]R&D, Sekisui Medical Co., ADME & Toxicology . Research Institute, Tokai-mura, Ibaraki, Japan.* Sponsor: T. Miyaoka.

Phamacokinetic and pharmacodynamic properties of generic drugs are confirmed to be within an acceptable bioequivalent range compared with the brand name counterpart. Generic drugs are, therefore, considered to be identical in intended use, dose, dosing route, efficacy, and by extension, safety. Therefore, toxicity evaluation is not required for the application of generic drugs to regulation authorities. On the other hand, the anxiety about different safety characteristics due to the difference of impurities in excipients is pointed out by medical doctor, pharmacist and so on. As a model study to evaluate the equivalency of risk of hepatotoxicity in humans, we examined changes in hepatic gene expression induced by two different formulations of methotrexate using chimeric PXB-mice, in which more than 70% of hepatic parenchymal cells are replaced by human hepatocytes. This animal model, which mimics human-type drug metabolism, has a potential to bridge the gap between rodent-type and human-type livers and to explain the difference of in vivo and in vitro responses of human hepatocytes against hepatotoxicants. The content of RHEUMATREX® CAPSULES 2mg (Pfizer Japan Inc.) or METHOTREXATE CAPSULES 2mg "TOWA" (Towa Pharmaceutical Co., Ltd.) was suspended in 0.5% methylcellulose and orally administered to PXB-mice three-times daily at a dose of 5 mg/kg, followed by hepatic total RNA preparation and gene expression analyses using GeneChip® Human Genome U133 plus 2.0 Array (Affymetrix Inc.). The equivalency of changes in hepatic gene expression induced by two different methotrexate formulations will be discussed in 18 biological pathways (such as NRF2-mediated oxidative stress pathway, CAR/PXR-mediated drug metabolism enzymes, fatty acid metabolism pathways) in addition to the global changes in hepatic gene expression.

PS 806 PRECLINICAL ASPECTS OF BIOSIMILAR DEVELOPMENT – PRACTICAL CONSIDERATIONS.

P. Baldrick and R. Donninger. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: S. Kirk.

Current regulatory thinking, notably in guidance issued in Europe, indicates that preclinical testing is necessary as part of the evaluation to show comparability for a biosimilar drug with the innovator product. Examination of publicly available data for the 13 biosimilars approved in Europe indicate fairly limited to more extensive preclinical testing depending on the nature of the drug class. This poster will discuss the process of determining what testing is needed for new biosimilars in development and practical considerations when performing this work. Considerations include the relevant biological and pharmacological studies and, for toxicology studies, the relevant species of use, study design and comparator requirements all with the aim of showing comparability but with unnecessary animal use in mind.

PS 807 THE INCIDENCE OF TOXICOKINETIC CONTROL SAMPLE CONTAMINATION IN REGULATORY TOXICOLOGY STUDIES.

S. Cahill and R. Turcan. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: S. Kirk.

The EMEA issued regulatory guidelines, in 2005, on the evaluation of control toxicokinetic samples in non clinical safety studies. These guidelines require the source of control toxicokinetic contamination to be investigated and if possible identified as any significant levels can lead to a study becoming invalid. It is difficult to find recent literature examples reporting the incidence of toxicokinetic control sample contamination in regulatory toxicology studies however, this investigation, into non-clinical studies conducted at Covance and also at other laboratories, identified control sample contamination in 38% of the studies. In the majority of these studies the contamination found was at a very low level in sporadic samples. The level of contamination was similar irrespective of whether the toxicology studies were performed at Covance (33%) or elsewhere (39%). The highest percentage of control sample contamination occurred in dog (56% of studies), with rat and primate each having around 40% of studies showing contamination. The dose route was found to influence the extent of contamination, with the lowest levels found in studies dosed by the intravenous route (22%) and the highest in the studies undertaken either by inhalation or subcutaneous administration (60%). There was also a higher incidence of contamination in studies of longer duration (52% of studies of 13-39 week duration) compared with those of shorter duration (22% of studies of 4 weeks or less). The incidence of contamination in embryo-foetal development studies was similar to that seen in studies of 4 weeks duration. The significance of this contamination to the overall integrity of the study and criteria for assessing this will be discussed. The importance of adequate control measures to help minimise or avoid control sample contamination will be considered.

PS 808 PHARMCOKINETICS AND 14-DAY TOXICITY OF INHALED BREVENAL IN CD-1 MICE.

G. Wang[1], D. G. Baden[2], D. C. Esparza[1], T. H. March[1], P. J. Kuehl[1] and J. M. Benson[1]. *[1]Lovelace Respiratory Research Institute, Albuquerque, NM and [2]Center for Marine Science, University of North Carolina Wilmington, Wilmington, NC.*

Brevenal, produced by the marine dynoflagellate, *Karenia brevis* has demonstrated high potency in preventing chemical-induced airway constriction and in enhancing tracheal mucus velocity in sheep. To further explore development of brevenal as a potential human therapeutic, the pharmacokinetics and potential toxicity of inhaled brevenal were evaluated in CD-1 mice. Pharmacokinetic analysis of lung and plasma was performed in male mice exposed to 275 or 1175 ng/L brevenal aerosol for 15 minutes. Brevenal was detectable from 0 to 120 minutes in the lungs with a dose dependent Area Under the Curve (AUC). Lung clearance $T_{1/2}$ for the low and high doses was 115 and 52 minutes, respectively. No brevenal was detected in the plasma at any time point up to 24 hours. In the subacute toxicity study, four groups of 22 (11 M/11F) mice were exposed to brevenal aerosol at an average of 273, 148, 43 and 0 ng/L for 15 minutes per day for 14 days. 6M/6F animals from each group were euthanized at day 15 (1 day after the last exposure), and the other 5M/5F animals euthanized at day 29. Brevenal inhalation had no significant effect on body

and organ weights, clinical signs and pathology, and major organ histopathology. Since the deposited doses used in this study are assumed to be over 1000 times higher than the intended dose for human clinical use, no local respiratory or systemic toxicity suggested development of brevenal as a human therapeutic is promising. (Research funded by NIEHS P01 ES10594)

PS 809 NONCLINICAL SAFETY ASSESSMENT OF AN ANTITHROMBOTIC THERAPEUTIC PROTEIN ON FERTILITY AND EMBRYO-FETAL DEVELOPMENT.

V. H. Chen[1], S. Jacobs[1], H. Ulrichts[1], S. Priem[1], S. Rossenu[1], J. Leuschner[2], J. Baumeister[1] and J. Holz[1]. *[1]Ablynx, Zwijnaarde, Belgium and [2]LPT, Hamburg, Germany.*

Ablynx develops therapeutic proteins, Nanobodies® (NB) for the use in patients affected by various diseases including cardiovascular, oncology and inflammation. They are based on the smallest functional fragments of naturally-occurring heavy-chain antibodies. The anti-vWF therapeutic protein (ALX-0081) is a humanized, bivalent NB that targets the platelet adhesive von Willebrand Factor (vWF). vWF is critically involved in a variety of thrombotic pathologies, such as platelet adhesion to areas of vascular damage in ACS and platelet string formation in patients with TTP. The anti-vWF NB specifically blocks interaction of the A1-domain in vWF with the platelet receptor GPIb-IX-V. Therefore, this small protein could be a powerful inhibitor of the pathophysiology of thrombotic events in ACS and TTP. The anti-vWF NB entered two distinct Phase II clinical development pathways as adjunctive treatment in the acute intervention in ACS and as adjunct to plasma exchange for the prolonged treatment of TTP. For nonclinical safety assessment, monkey and guinea pig were identified as the relevant models. This was confirmed via in vitro binding/efficacy and in vivo PK/PD measurements. The assessment includes a fertility evaluation built in a 3-month toxicity studies in monkey and an embryo-fetal toxicity study in guinea pig. The high dose was based on an anticipation of maximum target saturation guided by a mechanistic PK model of clearance mechanisms and drug-target relationships. ALX-0081 exhibits no sign of fertility effects in monkey. In guinea pig, maternal to fetus transfer of ALX-0081 was demonstrated with no toxic effects on embryo-fetal development. Interspecies differences need to be taken account in the assessment of placental transfer of monoclonal antibodies (mAb) mediated via FcRn receptors and small proteins without a Fc region. The potential of small proteins comparable in size to ALX-0081 which contains no Fc region to cross placental barriers from human, monkey or rodent is reviewed.

PS 810 TOPIRAMATE AND BIRTH DEFECTS: AN UPDATE.

J. M. DeSesso[1], A. Lavin Williams[1] and G. Koren[2]. *[1]Exponent, Alexandria, VA and [2]The Motherisk Program, The Hospital for Sick Children, Toronto, ON, Canada.*

Topiramate (TPM) has been associated with cleft palate in mice and limb defects in rats and rabbits. To assess the relevance to humans of these findings, both animal and human data of gestational exposure to TPM were evaluated. Analysis of animal data used to support FDA approval shows that cleft palate was observed in both the control and TPM-treated groups in a mouse developmental study, but the incidence for all groups was within the historical control range. Further, mice are predisposed to cleft palate. More importantly, neither rats nor rabbits exhibited elevated cleft palate in response to gestational TPM treatment, although both species showed a small increase in limb defects at doses much higher than those associated with cleft palate in the mouse. In rodents, ectrodactyly (reduced number of digits) has been shown to occur due to developmental carbonic anhydrase inhibition. Primates do not appear to be susceptible to limb malformations due to in utero carbonic anhydrase inhibition. To evaluate the human data for cleft palate, Medline and Pubmed were reviewed from 1996 to July 1, 2010 for original cohort studies of first trimester exposure to TPM, where the authors followed pregnancy outcome and reported rates of major malformations. The comparison group consisted of all original papers that followed cohorts of pregnant, untreated epileptic women and reported on rates of major malformations. Four registries involving TPM treatment and meeting the inclusion criteria were identified from Israel, North America, Britain, and Australia, with a total of 406 pregnancies and a mean rate of major malformations of 3.7% (95% CI=1.9-5.5%). In the comparison group (12 registries, reporting on a total of 710 pregnancies), the mean rate of major malformations of 3.4% (95% CI=1.9-4.8%). The risk ratio of major malformations associated with TPM was 1.09 [95% CI=0.58 – 2.06]. In conclusion, both the animal and human data indicate that fetuses exposed in utero to TPM should not be at increased risk for major malformations including cleft palate or limb defects.

PS 811 LONG-TERM PULMONARY SAFETY ASSESSMENT OF AFREZZA™ IN RATS AND DOGS.

S. Greene[1], K. Nikula[2], J. Reynolds[3], D. Poulin[4], K. McInally[5], D. Townson[1] and P. Richardson[1]. *[1]MannKind Corp, Valencia, CA, [2]Seventh Wave Laboratories, Chesterfield, MO, [3]J.A. Reynolds & Associates, Madison, CT, [4]Charles River Laboratories, Montreal, QC, Canada and [5]ITR Laboratories, Montreal, QC, Canada.*

The inhalable insulin Afrezza™ was evaluated in nonclinical safety studies as a New Molecular Entity (NME) for diabetes treatment. Chronic inhalation and carcinogenicity studies were conducted in Sprague Dawley rats and a chronic inhalation study was conducted in Beagle dogs. Daily doses of either the novel excipient Technosphere® (fumaryl diketopiperazine) particles or various doses of Afrezza™ (insulin adsorbed to Technosphere particles) were administered by the nose-only (rats) or oronasal (dogs) routes. Tissues were evaluated by histopathology and respiratory cell proliferation was assessed by immunostaining of proliferating cell nuclear antigen (PCNA). There were no test article-related changes in the laryngopharynx, larynx, trachea, bronchi or lung of rats. Test article and Technsophere particle findings in the nasal cavity of rats were limited to the presence of eosinophilic globules in the olfactory and respiratory epithelium that were considered nonspecific responses to chronic, high level administration of particulate materials. After 39-wks in dogs, there was a low incidence of minimal to mild alveolar and/or bronchial interstitial neutrophil infiltrate in the lungs at the high Afrezza™ dose. This cellular infiltrate regressed completely during the 8-wk recovery period. Based on evaluation of H&E stained tissues, there was no evidence of amyloid deposition in any portion of the respiratory tract in either species. Inhalation of Afrezza™ up to 104 wks in rats and 39-wks in dogs did not increase PCNA labeling in alveoli, large bronchiolar or terminal bronchiolar tissues. Based on no test article-related adverse findings in the lungs or increases in cell proliferation in rats or dogs, the no adverse effect level was established in both species as the highest studied doses. Chronic inhalation of Afrezza™ was well tolerated and supports the safe use of this NME in humans over extended periods.

PS 812 TOXICOLOGICAL EVALUATION OF LMI1195, A NOVEL SYMPATHETIC NERVOUS SYSTEM PET IMAGING AGENT.

M. Mistry[1], Y. Tang[1], D. Onthank[1], E. Cheesman[1], K. Baghdadi[2], S. Watson[3], K. Beard[3] and S. Robinson[1]. *[1]Lantheus Medical Imaging, North Billerica, MA, [2]Analytical Chemistry, Charles River Laboratories, Senneville, QC, Canada and [3]ITR Laboratories, Baie d'Urfé, QC, Canada.* Sponsor: W. Ruddock.

LMI1195, a benzylguanidine derivative, acts as a novel norepinephrine transporter (NET) ligand and when labeled with 18F, can be used as a radiotracer for mapping the nerve terminals in vivo. Toxicological studies were performed with the non-radioactive form ([19F]LMI1195) formulated in the clinical vehicle to support the development as a cardiac neuronal PET imaging agent. Single dose IV administration of LMI1195 in the Sprague Dawley (SD) rats and the Beagle dogs up to 0.5 mg/kg was not associated with clinical signs, changes in food intake, body weight, clinical and gross pathology or histopathology observed over 14 days. Repeat daily IV injections over 28 days in either SD rats or Beagle dogs with LMI1195 at 0.005, 0.05 and 0.5 mg/kg/day produced dose related exposure but no treatment related effects (clinical, gross or histopathological changes) in either species. Toxicokinetic analysis on Day 1 and 28 indicated Cmax increased at least proportional to dose, rapid blood clearance, a large volume of distribution and no marked or consistent gender differences, minimal (rat) to some drug accumulation (dog) for the 28-day treatment period. Injections via IV and perivascular routes in the rabbit ear with concentrations up to 500 μg/mL of LMI1195 produced no irritation beyond vehicle control. In vitro testing with LMI1195 up to 100 μg/mL indicated no hemolysis. Genotoxicity tests included a bacterial mutation assay, human lymphocyte chromosome aberration assay and rat micronucleus assay up to the maximal concentration or tolerated dose levels. Overall these genetic toxicity studies demonstrated that LMI1195 lacks genotoxic potential. Based on these findings, the no-observable-adverse-effect-level (NOAEL) in both the rat and dog is 0.5 mg/kg for LMI1195 that provides a large safety margin based on body mass (~1800x) and BSA (~300x) relative to the anticipated clinical dose (≤0.28 μg/kg) for imaging studies.

S 813 EPIGENETICS, METALS, AND CANCER.

M. Costa. *Department of Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

Mounting experimental data indicates that epigenetic programs of gene expression are altered following exposure to carcinogenic chemicals. Carcinogenic metals such as nickel, chromate, and arsenite are excellent examples of agents that cause cancer

and induce alterations in epigenetic marks. To better understand these alterations, it is important to address the mechanisms of how metals alter epigenetic programs at a variety of levels ranging from inhibition of histone demethylases, miRNA, DNA methylation, and histone acetylation. Although the focus is mostly on cancer other toxicities can also be manifested as a result of these epigenetic effects. Thus, we will being by presenting recent evidence that sustained exposure to low environmental doses of chromium leads to gradual changes in histone marks and signal transduction pathways that cumulatively affect gene expression, silencing expression of tumor suppressor genes and of PAH-induced detoxification genes. We will then examine miRNA and protein-coding gene promoter microarrays to determine the extent and temporal nature of changes in DNA methylation and histone modification in a human urothelial cell model of arsenical-induced malignant transformation. Before concluding a member of the panel will demonstrate that dioxygenases such as the histone demethylases and DNA repair enzymes ABH2 are major targets of nickel ions in cells. Data will be presented that suggest that the alteration of H4K16 acetylation affects arsenic toxicity in both yeast and human cells. Finally, detailed studies on the epigenetic regulation of MT-3 expression in normal, tumorigenic and metal-transformed cell types will be highligthed. Mt-3 expression is repressed in normal bladder and breast cells but increased in expression in the respective tumors. In summary, we will detail state of the art methodology that examines the epigenetic mechanisms of metal carcinogenesis.

S 814 CHROMIUM-INDUCED CHROMATIN REMODELING.

A. Puga. *Department of Environmental Health, University of Cincinnati, Cincinnati, OH.*

Hexavalent chromium exposure increases the risk of leukemia and tumors of lung, liver, kidney, bladder, bone, prostate and stomach. Studies also show non-cancer health effects on the respiratory, GI tract, immune system, liver, and kidney. Frequently, chromium coexists in complex mixtures with polycyclic aromatic hydrocarbons (PAH) such as benzo[a]pyrene (BaP). The long-range goal of our research has been to develop an understanding of mechanisms responsible for the adverse health effects of exposure to binary mixtures of BaP and chromium(VI) by exploring the hypothesis that environmentally relevant binary mixtures of chromium(VI) and BaP have a synergistic effect on toxicity and carcinogenicity. We showed that high-dose acute chromium treatment interferes with the assembly of productive transcriptional complexes by cross-linking HDAC1/DNMT1 complexes to chromatin and inhibits phosphorylation, acetylation and methylation marks established by BaP-induced gene transactivation in histones H3 and H4. These changes inhibit recruitment of RNA polymerase II to the Cyp1a1 promoter, block BaP-inducible gene expression and stimulate the formation of BPDE-DNA adducts. On the other hand, sustained exposure to low doses of hexavalent chromium leads to gradual changes in histone marks and signal transduction pathways that cumulatively affect gene expression and silencing by epigenetic mechanisms. Specifically, environmentally relevant doses of chromium(VI) have opposite effects on the BaP-mediated induction of CYP1A1, CYP1B1 and CYP1A2 cytochrome P450 genes, repress tumor suppressor genes and induce expression of pro-apoptotic genes. Epigenetic modification of tumor suppressor gene promoters has been found in chromium-exposed workers who developed lung cancer suggesting alternative mechanisms for chromium to target cancer development. Knowledge derived from our research may have a major impact on the medical translation of epidemiological findings and, by identifying molecular targets useful to reduce disease incidence, may significantly contribute to the development of therapeutic and preventative measures.

S 815 EPIGENETIC REMODELING BY ENVIRONMENTAL ARSENICALS.

B. W. Futscher. *Department of Pharmacology & Toxicology, The University of Arizona, College of Pharmacy, Tucson, AZ.*

Environmental exposures to arsenicals are a significant health concern to human populations, since they are widely distributed in the environment and are linked to the etiology of human disease, including bladder cancer. To evaluate the hypothesis that arsenicals induce malignant transformation via disruption of epigenetic control, we used human miRNA and protein-coding gene promoter microarrays to determine the extent and temporal nature of changes in DNA methylation and histone modification in a human urothelial cell model of arsenical-induced malignant transformation. Results show that arsenical-induced malignant transformation results in non-random aberrant changes in the DNA methylation and histone modification state of transcriptional regulatory regions of both miRNA genes and protein-coding genes that, in turn, are linked to changes in the expression of the associated gene. These complex epigenetic changes are coincident with and participate in the acquisition of malignant properties, such as anchorage independent growth and tumor formation in immunocompromised mice. These arsenical-induced DNA methylation and histone modification changes are stable, since malignantly transformed cells removed from exposure to the transforming arsenical exhibited no reversion in DNA methylation or histone modification state, associated gene expression, or malignant phenotype. These data suggest that arsenicals act as epimutagens and directly link their ability to induce malignant transformation to their actions on the epigenome.

S 816 OXIDATIVE HISTONE DEMETHYLASES AND ABH2 DNA DEMETHYLASE AS TARGETS OF NICKEL IONS.

M. Costa. *Department of Environmental Medicine and Department of Pharmacology, New York University School of Medicine, New York, NY.*

Iron- and 2-oxoglutarate-dependent dioxygenases are a diverse family of nonheme iron enzymes that catalyze various important oxidations in cells. A key structural motif of these dioxygenases is a facial triad of 2-Histidines-1-Carboxylate that coordinates the Fe(II) at the catalytic site. Using histone demethylase JMJD1A that demethylates H3K9 dime and DNA repair enzyme ABH2 as examples, we show that this family of dioxygenases is highly sensitive to inhibition by carcinogenic nickel ions. We find that, with iron, the 50% inhibitory concentrations of nickel (IC50[Ni(II)]) are 25 μM for JMJD1A and 7.5 μM for ABH2. Without iron, JMJD1A is ten times more sensitive to nickel inhibition with an IC50[Ni(II)] of 2.5 μM and approximately one molecule of Ni(II) inhibits one molecule of JMJD1A, suggesting that nickel causes inhibition by replacing the iron. Using XAS, we demonstrate that nickel binds to the same site in ABH2 as iron and replacement of the iron by nickel does not prevent the binding of the cofactor 2-oxoglutarate. Finally, we show that nickel ions target and inhibit JMJD1A in intact cells, and disruption of the iron-binding site decreases binding of nickel ions to ABH2 in intact cells. We will also discuss the role of nickel and hypoxia in the inhibition of the JARID-1 family that oxidatively demethylate H3K4 trimethylation. This results in increases in global H3K4 trimethylation which has been mapped using ChIP-Seq.

S 817 YEAST FUNCTIONAL GENOMICS REVEAL ROLE FOR ARSENICALS IN HISTONE ACETYLATION.

C. Vulpe[1], W. Jo[1], F. Chu[4], X. Ren[2], A. Burlingame[3], M. Smith[2] and L. Zhang[2]. [1]*Nutritional Science and Toxicology, University of California, Berkeley, Berkeley, CA,* [2]*School of Public Health, University of California, Berkeley, Berkeley, CA,* [3]*Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA and* [4]*Molecular, Cellular & Biomedical Sciences, University of New Hampshire, Durham, NH.*

Arsenic is a human toxicant and carcinogen commonly found as a contaminant in drinking water. Arsenite (AsIII) is the most toxic inorganic form, but recent evidence indicates that the metabolite monomethylarsonous acid (MMAIII) is even more toxic. We have used a chemical genomics approach to identify the genes that modulate the cellular toxicity of MMAIII and AsIII in the yeast Saccharomyces cerevisiae. Multiple subunits of multimeric complexes including SAS, SAGA, SWR1 and Itc1p-Isw2p, associated with chromatin remodeling processes were identified as involved in response to both arsenicals. We focused further study on the SAS complex. The human homolog of SAS2, MYST1, encodes a histone 4 lysine 16 (H4K16) acetyltransferase. Additional support for the involvement of H4K16 acetylation in AsIII toxicity came from the finding that the H4K16→R yeast mutant strain, which contains a non-acetylatable arginine residue instead of the regular lysine, exhibited slow growth compared to the wild type and was sensitive to high doses of AsIII. Silencing of MYST1 in human uroepithelial UROtsa cells increased their sensitivity to AsIII and to its more toxic metabolite monomethylarsonous acid (MMAIII) at doses relevant to high environmental human exposures. In addition, both AsIII and MMAIII treatments decreased global H4K16 acetylation levels in a dose- and time-dependent manner. Together, these data suggest that the alteration of H4K16 acetylation affects arsenic toxicity in both yeast and human cells.

S 818 THE ROLE OF EPIGENETICS IN THE INDUCTION OF METALLOTHIONEIN-3 DURING BLADDER AND BREAST TUMORIGENESIS.

S. Garrett. *Department of Pathology, University of North Dakota, School of Medicine & Health Sciences, Grand Forks, ND.*

Metallothionein-3, a member of the classic heavy metal-binding metallothionein gene family, is expressed mainly in the brain and at lower levels in the epithelial cells of the kidney. In breast and bladder epithelial cells, MT-3 expression is repressed,

yet is induced in most bladder and breast tumors with correlation of expression to the grade of the tumor in bladder cancer and a correlation to unfavorable clinical outcome in breast cancer. In non-tumorigenic breast and bladder cell lines, MT-3 expression is also repressed and refractory to metal induction; however, during tumorigenesis, the gene transitions from a dormant state, to an actively transcribed gene. Initial investigations into the mechanism of oncogenic activation of MT-3 was fraught with difficulty due to the inability of MT-3 promoter-reporter constructs to model expression in normal and transformed cell types. Using the epigenetic modifying agents,5-azacytidine, and the histone deacetylating agent, MS-275, the MT-3 gene can be activated in non-tumorigenic epithelial cells. The resulting activated state allows for metal induction of MT-3 through the metal-regulatory elements (MREs), previously thought to be inactive in MT-3 gene regulation in the brain. This talk will detail studies on the epigenetic regulation of MT-3 expression in normal, tumorigenic and metal-transformed cell types.

S 819 HUMAN VARIABILITY IN SUSCEPTIBILITY TO ENVIRONMENTAL TOXICANTS.

H. Mortensen. *NCCT, U.S. EPA, Research Triangle Park, NC.*

Defining the differing levels of susceptibility across human populations in response to environmental chemicals can provide information to define population risk factors and in turn, allow for risk levels to be based on the most susceptible populations. Data from high-throughput/high content (HT/HC) technologies, including 'omics, have been integral in the identification and characterization of drug-target or disease loci and have the potential to be informative for characterizing the effects and dose-response assessment of chemical exposure and outcomes within genetically heterogeneous populations. Many of the same 'omics technologies have been successfully utilized to provide data that informs the mechanism of action for environmental chemicals, including the identification of perturbed toxicity pathways. In addition, large scale population genotyping studies, such as the HapMap, can help to establish levels of variability at chemical-associated, target loci across human populations, and in comparison to genome-wide patterns. Individual genotype-phenotype combinations can then be verified using in vitro methods, or extrapolation from animal models. This session includes talks on some of the latest approaches to informing population variability and the identification of susceptible populations through the use of HT/HC data, particularly from genomics technologies. We will discuss how 'omics data in combination with data from enhanced animal models, publically available datasets and related computational tools can be used to identify biomarkers, and subsequently define risk for genetically heterogeneous populations, and how this variability translates to human risk and progression of disease.

S 820 POPULATION-BASED DISCOVERY OF TOXICOGENOMICS BIOMARKERS FOR HEPATOTOXICITY.

I. Rusyn. *Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, Chapel Hill, NC.*

Current approaches to biomarker discovery through gene expression profiling usually utilize a single or few strains of rodents, limiting the ability to detect biomarkers that may represent the wide range of toxicity responses typically observed in genetically heterogeneous human populations. To enhance the utility of animal models to detect response biomarkers for genetically diverse populations, a genetically-defined panel of mouse strains can be used. By taking into the account strain-specific gene expression patterns it is possible to establish genetic polymorphism-dependent and -independent pathways perturbed by the toxicants. Since genetic regulation of gene expression is a key contributor to population diversity, better understanding of the relationship between toxicity responses and the individual's genetic background is needed.

S 821 EXPERIMENTALLY DEFINING TOXICITY PATHWAYS USING *IN VITRO* HIGH-CONTENT SCREENING OF EMBRYONIC FIBROBLASTS FROM THE MOUSE DIVERSITY PANEL.

R. Thomas. *Genomic Biology and Bioinformatics, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

A key component of the vision laid out in the NRC report 'Toxicity Testing in the 21st Century' is the development of assays based on toxicity pathways. These toxicity pathways were defined as cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects. Despite the importance of these pathways, no strategy was put forth on how to identify them. In this presentation, we describe the use of primary embryo fibroblasts isolated from 32 genetically characterized inbred mouse strains to experimentally identify potential toxicity pathways. The mouse embryo fibroblasts were exposed to one of a set of diverse environmental and pharmaceutical agents at multiple doses and for two different exposure intervals. The effects of exposure were assessed using high-content screening assays for integrated cellular responses. Cross-strain differences in sensitivity to the different treatments allowed genetic mapping to be performed on each endpoint, resulting in a collection of candidate loci that underlie these cellular responses. Enrichment analysis on these candidate loci has identified a number of pathways that can serve as the basis for developing toxicity pathway assays.

S 822 HERITABILITY IN DOSE RESPONSE - PUTTING THE HORSE BEFORE THE CART.

A. Motsinger-Reif. *Department of Statistics, North Carolina State University, Raleigh, NC.* Sponsor: H. Mortensen.

The investigation of genetic and genomic factors that determine or predict differential response to environmental exposures is a crucial goal of toxicogenomics, and relies on the unstated and often untested assumption that this differential response is a heritable trait. While limitations in traditional genetic study designs often prohibit the estimation of the relative contribution of genetics to response traits in such data, new approaches may allow for such estimates. For example, we demonstrate an ex vivo model system to determine the heritability of drug-induced cell killing and performed genome-wide analysis for quantitative trait loci for prioritizing drug response outcomes. Such an approach could be useful in toxicogenomics for prioritizing response outcomes for genetic investigation.

S 823 ASSOCIATION OF GENETIC POLYMORPHISMS, MRNA EXPRESSION OF P53 AND P21 WITH CHRONIC BENZENE POISONING IN A CHINESE OCCUPATIONAL POPULATION.

N. Rothman. *Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.* Sponsor: H. Mortensen.

Dr. Nat Rothman of the NCI will describe his work using genomic approaches to study precursor and disease outcomes associated with benzene exposure in humans. Specifically, he will discuss (1) using genetic polymorphisms of white blood cell count (WBC) in a Chinese worker population to study the relationship between hematologic outcomes associated with benzene exposure and the disease pathway; (2) the toxicity pathway in periphera blood mononuclear cells for benzene using toxicogenomic approaches. He will discuss the relationship between these biomarkers and disease risk in the general population and the implications for risk assessment.

S 824 PERSONALIZED MEDICINE, INDIVIDUALIZED DRUG THERAPY: CAN THESE GOALS BE REALISTICALLY ACHIEVED?

D. W. Nebert. *Departments of Environmental Health & Pediatrics, Division of Human Genetics, University Cincinnati Medical Center, Cincinnati, OH.*

For each environmental toxicant, an identical exposure is well known to elicit a different response between virtually any two individuals. From this standpoint, human risk assessment is no different from "personalized medicine", or its subset, "individualized drug therapy". Can any number of DNA variant sites (genotype) ever be associated—-100% of the time—-with diagnosis of a complex disease such as benzene-induced leukemia (phenotype)? Can any genotype ever predict, 100% of the time, each patient's response to a metal, chemical or drug (phenotype)? Ideally, a 100% level of success in genotype-phenotype association studies is what physicians' desire for risk assessment, personalized medicine, or individualized drug therapy to be successful. Several researchers have insisted, however, that the genome is far too complex for us to understand at the present time.

Contributions by ENCODE, the HapMap Consortium, genome-wide association (GWA) studies, and "third-generation DNA deep sequencing" have given us valuable breakthroughs in understanding a few complex diseases, whereas many others have yielded puzzling results. Comparing "monogenic", high-penetrance predominantly monogenic (hPpM) toxicogenetic disorders, and "complex" diseases—-the percentage that one gene might contribute to the trait varies: >90%, 15%-25%, and 0.01%-1.0%, respectively. I advocate that adverse chemical reactions are not different from complex diseases.

Recent studies show that 14, 20 or 95 loci significantly associated (P <5 x 10–8) with complex traits such as prolonged-QT-interval, height, and lipid profile, respectively, explain 5.0-6.5%, <3%, and 25-30% of the genetic variance. Thus, the

contribution of epigenetics to complex disease phenotypes appears to be very large, i.e. between 70% and >97%. The interaction of Mendelian genetics (changes in DNA sequence) with Lamarckian genetics (no alterations in DNA sequence; i.e. epigenetics) will be emphasized in this overview presentation.

S 825 TOXICOLOGICAL CONSIDERATIONS IN THE GULF OF MEXICO OIL SPILL.

M. C. Madden. *ORD, NHEERL, HSD, Clinical Research Branch, U.S. EPA, Chapel Hill, NC.*

The Deepwater Horizon oil rig explosion on April 20, 2010 resulted in a release of petroleum crude oil into Gulf of Mexico waters. The release, 41 miles offshore, was about 1 mile in depth. Oil dispersants were employed to change surface oil and underwater properties. Surface oil was also combusted and physical barriers deployed. Oil was estimated to have spread to an estimated 2500 square mile in the Gulf. On July 15, the well head was capped reducing oil flow to a minimum. A number of exposure uncertainties have arisen as a result of the spill related to the locations the oil has dispersed to, including volatilization to the atmosphere; and the dose of contaminant reaching wildlife, humans, and vegetation in the affected areas, and physicochemical properties of the dispersant-treated and aged oil. We will present findings related to understanding the migration of the spill components, the habitats and organisms contaminated, and the characteristics of altered crude. Routes of exposure for humans to oil components will be presented in part based on data from other studied oil spills. Knowledge of these currently unsure parameters will assist in understanding the potential biological and health effects induced by the spill. The major possible ecological effects will be described and compared to the normal Gulf ecosystem dynamics. The choice of the specific oil dispersants utilized will be discussed with an emphasis on the toxicity assays employed; toxicity endpoints still not fully examined will be identified. The types and likelihood of possible human adverse health effects, both acute and latent, will be presented in light of what will be known of the exposure routes and the conditions (e.g. heat). In order to comprehend possible effects in a comprehensive manner in order to develop estimates of various risks, the data sources available to perform such an assessment will be identified (e.g., estimates of contaminated seafood consumption). Finally, data deficiencies needed for risk assessment will be described.

S 826 THE GULF OF MEXICO ECOSYSTEM: CONSEQUENCES OF THE BP OIL SPILL.

W. Benson, S. Jordan, R. Greene, V. Engle, M. Hemmer and M. Barron. *Gulf Ecology Division, U.S. EPA, Gulf Breeze, FL.*

The Gulf of Mexico, a semi-enclosed basin of the Atlantic Ocean, is bounded by 5 U.S. states, Mexico, and Cuba. Its shorelines mostly adjoin low-lying coastal plains, where extensive estuaries, marshes, barrier islands, and shallows provide outstanding habitats for fish, shellfish, and wildlife. Seaward, the broad continental shelf drops off to the abyssal zone, >3000 m deep, thus the Gulf is endowed with a full range of marine habitats. The abundance of physical habitats and a climate spanning warm temperate, sub-tropical, and tropical zones combine to make the Gulf one of the world's most productive bodies of water, a center of biodiversity, and home to major commercial and recreational fisheries. Since the April 2010 explosion of the Deepwater Horizon oil exploration platform, unprecedented quantities of crude oil have been released into the Gulf. Nearly 2 million gallons of oil dispersant have been applied. It is far too early to know the total damage to the Gulf's ecosystem, but comprehensive baselines of data are available for estuaries, coastal waters, and wetlands as a result of surveys conducted by EPA and partners. An EPA report was prepared in response to the disaster that summarizes historical and immediate (2010) ecological baseline conditions for Gulf estuaries, including water and sediment quality, fish tissue contaminants, and other indicators. This baseline will be used for comparison with current and future monitoring data to detect changes in condition. Additional reports by EPA examined the acute toxicity of dispersants, oil, and dispersant-oil mixtures on sensitive Gulf aquatic species, but the full extent of toxicological effects on aquatic species and other wildlife remain unknown. The best case scenario for the Gulf ecosystem is that the major effects of the oil disaster will be local in extent and short-lived. The worst case is that pervasive effects, especially chronic toxicity, will be widespread, enduring, and disruptive of reproduction and survival for fish, invertebrates and wildlife.

S 827 THE FATE, BEHAVIOR, AND WEATHERING OF SPILLED OIL FROM THE DEEPWATER HORIZON SPILL.

J. Michel. *Research Planning, Inc., Columbia, SC.* Sponsor: M. Madden.

The oil from the Deepwater Horizon spill had unique fates,, behaviors, and weathering patterns because of the release from 5,000 feet below the water surface and the chemical composition of the oil. However, the oil did follow expected patterns based on our understanding of oil behavior in the environment. These patterns of oil fate and behavior affect the toxicity of the oil, both to water column and shoreline resources. An understanding of these properties would help predict the possible toxicity of the pollutant and what species would potentially be exposed. This presentation will discuss the physicochemistry & fate of oil in seawater, with some emphasis on the type of oil spilled, weathering changes in different environments (water column, water surface, and shoreline), pathways of exposure, the habitats that were affected, and the different response options used to reduce overall impacts.

S 828 OIL SPILL CHEMICAL DISPERSANTS: THE GOOD, THE BAD, OR THE UGLY?

C. L. Mitchelmore. *Center for Environmental Science, University of Maryland, Solomons, MD.* Sponsor: M. Madden.

The use of chemical dispersants following an oil spill event has always been a controversial issue. Their use during the Deepwater Horizon oil leak has again raised issues regarding the numerous data gaps and uncertainties on their impacts on organisms, especially in the long-term, to many understudied and sensitive taxa (e.g. corals). Their use in this specific incident has brought into question further issues given the extent of their use (1.8 million gal. over 3 months) and their novel use directly at the wellhead 5000 ft underwater. During this spill intense scrutiny was placed on the specific types of dispersants being using (Corexit 9527 and 9500A) and calls for using less toxic alternatives or stopping altogether were heavily reported issues. This presentation will give an overview on what is and isn't known about the toxicity of the chemical dispersants listed on the National Contingency Plan Product Schedule, including an analysis of past and current data sets and an overview of some of the limitations regarding these toxicity tests. The presentation will evaluate whether enough sufficient toxicity data exists to make rationale decisions about their use, and what are the major uncertainties and data gaps. We have recently attempted to fill some of these data gaps in understudied, sensitive taxa, by exposing corals to oil (weathered Arabian Light crude oil), dispersant (Corexit 9500A) and chemically-dispersed oil using environmentally realistic, controlled and fully-chemically characterized laboratory test solutions. In comparison to other species, our data shows that the soft coral (Xenia elongata) is impacted at the population level by short-term (8hr), low-levels (ppm) of Corexit 9500A and chemically-dispersed oil. Exposure to oil components occurred from both the dissolved and particulate oil phases resulting in delayed mortality and a variety of sub-lethal biological effects. We recommend that further experiments should characterize the response of hard coral species and in the meantime careful trade-off decisions should be made when considering dispersant use in or near a coral reef.

S 829 SHORT-TERM HEALTH EFFECTS FROM OIL SPILLS.

N. Sathiakumar. *University of Alabama at Birmingham, Birmingham, AL.* Sponsor: M. Madden.

The Gulf of Mexico oil spill is the largest accidental release of oil into marine waters causing considerable concern on the potential human health effects from exposure to the massive quantities of spilled oil and the chemical dispersants used in cleanup operations. Much of the knowledge on human health effects following exposure to spilled oil comes from epidemiologic studies of supertanker oil spills. Of 38 supertanker spills since 1980s, only 7 have been studied. The available studies provide consistent evidence of short-term toxic symptoms involving ocular, respiratory and neurological systems and of psychological symptoms among exposed community members and among cleanup/volunteer workers. In addition, certain subgroups of cleanup/volunteer workers had a high frequency of dermal symptoms besides other toxic symptoms, ergonomic symptoms such as back pain and work-related injuries. Durations of employment in cleanup operations was identified as a risk factor. The use of personal protective equipment (PPE) was less than optimal among volunteer workers. Poorly-informed workers on PPE use had an increased risk of exposure. Selected biochemical measurements for kidney and liver functions and/or spirometry for lung function among exposed community members, subgroup of children and cleanup workers were within normal limits. One study suggests that genotoxic and endocrine function alternations may be related to exposure, whereas another study found no evidence of genotoxicity damage. In conclusion, the short-term health effects of oil spills are primarily acute toxic symptoms and impairment of psychological health. No firm conclusion can be drawn on systemic effects or of genotoxicity as the relevant data are available only at a single point in time. Considerable uncertainty remains with regard to the potential for long-term health effects. Identification of the magnitude of the short-term effects will assist in devising strategies to limit exposures and control adverse health outcomes, particularly among vulnerable populations. Long-term biomonitoring studies of high risk populations are needed to evaluate the potential long-term health effects following oil spill-related exposures.

S 830 RISK ASSESSMENT OF THE SPILL: CRITICAL INFORMATION NEEDS.

W. H. Farland. *Colorado State University, Fort Collins, CO.*

The Gulf oil spill has resulted in efforts to understand pathways to human exposure and risk. Nonetheless, the situation in the Gulf area continues to be uncertain. Understanding the implications of these "real world" exposures requires better and different data and innovative tools and approaches. Data are needed to evaluate fate and transport of oil, associated chemicals and dispersants from source to receptor. Monitoring and modeling tools are needed to address these potential toxicants in all environmental media and in the food supply. Federal agencies have stepped up monitoring of air, water and Gulf seafood. Examples of such data in the region include air sampling for particulates and volatile organics; sampling of the Gulf and of fresh water systems; seafood sampling; and evaluation of utilization of personal protective equipment (PPE). Additional understanding of routes of exposure could come from sampling of wells around disposal sites for oily debris; creel surveys; and monitoring of game animals. In addition, an understanding of both toxicokinetics and toxicodynamics of oil, volatile organics, products of incomplete combustion and dispersants will be needed to effectively assess hazard and risk. Some of the challenges have been a lack of understanding of the impacts of aggregate and cumulative exposure and additivity to preexisting backgrounds of exposure and disease. In addition, understanding potential risk will require development and application of methods to address uncertainty and variability of exposure and of population response. Assessment of impacts on children and the elderly; ethnic and tribal populations with specific behaviors and individuals who may be genetically pre-disposed to respond in an adverse way represent additional challenges. Such data will assist in developing approaches to continued exposure prevention and disease surveillance. Because of the magnitude of the release and the complexity of the situation, data collection and tool development must be comprehensive and long-term for satisfactory assessment of toxicologic and public health implications of the Gulf oil spill.

S 831 TRANSLATIONAL TOXICOLOGY: MOLECULES TO GLOBAL HEALTH.

T. W. Kensler[1, 2] and J. D. Groopman[2]. [1]*Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, PA and* [2]*Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

The appreciation that the environment plays a universal role in human health has evolved rapidly in recent decades, drawing largely on new analytic technologies, advanced data acquisition, informatics and modeling, mechanistic studies in toxicology, and the conceptual framework of risk assessment. To adequately explore rapidly evolving issues, we will highlight four case studies detailing exposures through the vectors of air, food and water to agents, natural and anthropogenic that impact health across the lifespan of millions of people across the globe. Specific attention will be given relative to the use of toxicologic and epidemiologic data with improved techniques for quantifying exposure of biologically effective dose produce estimates of risks from environmental hazards to selected target populations. The global health implications of these exposures and possible intervention strategies to mitigate these exposures and their consequent health effects will also be discussed. In conclusion, the panelists will discuss the current status of toxicology and global health and a consideration of the challenges emerging in the next 50 years.

S 832 MYCOTOXINS: IMPACTS ON CANCER AND CHILDREN'S HEALTH.

C. P. Wild. *International Agency for Research on Cancer, Lyon, France.* Sponsor: T. Kensler.

Each year some 4.5 million children die in sub-Saharan Africa before their fifth birthday. Many of these deaths are due to infectious diseases, with an underlying susceptibility created by chronic malnutrition and growth faltering. Our previous work revealed a striking association between aflatoxin biomarkers and impaired growth in West African children. Thus, aflatoxins and other mycotoxins may contribute significantly to child morbidity and mortality. Collectively, the role that these mycotoxins play during this critical period of exposure in early life is lacking and the mechanisms of growth impairment are not understood. Biomarkers of these exposures and resultant risks, initially developed for evaluating the role of aflatoxin in the etiology of liver cancer in humans, will be essential to dissect this biology and this will form a vital foundation for development of a rationale for prevention strategies.

S 833 ARSENIC: RECENT FINDINGS FROM THE HEALTH EFFECTS OF ARSENIC LONGITUDINAL STUDY IN BANGLADESH.

J. H. Graziano. *Environmental Health Sciences, Columbia University, New York, NY.*

The contamination of groundwater by arsenic in Bangladesh is a major public health concern affecting between 35-75 million people. Although it is evident that high levels (>300 microg/L) of arsenic exposure from drinking water are related to adverse health outcomes, health effects of arsenic exposure at low-to-moderate levels (10-300 microg/L) are not well understood. We established the Health Effects of Arsenic Longitudinal Study (HEALS) with more than 20,000 men and women in Araihazar, Bangladesh, to prospectively investigate the health effects of arsenic predominantly at low-to-moderate levels (0.1 to 864 microg/L, mean 99 microg/L) of arsenic exposure. Findings to date suggest adverse effects of low-to-moderate levels of arsenic exposure on the risk of pre-malignant skin lesions, high blood pressure, neurological dysfunctions, and all-cause and chronic disease mortality. In addition, the data also indicate that the risk of skin lesion due to arsenic exposure is modifiable by nutritional factors, such as folate and selenium status, lifestyle factors, including cigarette smoking and body mass index, and genetic polymorphisms in genes related to arsenic metabolism. The analyses of biomarkers for respiratory and cardiovascular functions support that there may be adverse effects of arsenic on these outcomes and call for confirmation in large studies. A unique strength of the HEALS is the availability of outcome data collected prospectively and data on detailed individual-level arsenic exposure estimated using water, blood and repeated urine samples. Future prospective analyses of clinical endpoints and related host susceptibility will enhance our knowledge on the health effects of low-to-moderate levels of arsenic exposure, elucidate disease mechanisms, and give directions for prevention.

S 834 SECONDHAND TOBACCO SMOKE: USING BIOMARKERS TO INFORM PUBLIC POLICY.

S. S. Hecht. *Masonic Cancer Center, University of Minnesota, Minneapolis, MN.* Sponsor: T. Kensler.

During smoking of cigarettes and other tobacco products, a stream of smoke is released between puffs into the air. This sidestream smoke is mixed with exhaled mainstream smoke and air in the environment to form secondhand smoke. Secondhand smoke is an accepted cause of lung cancer in non-smokers. Specific tobacco smoke biomarkers have been crucial in establishing this relationship. The most effective and widely applied biomarkers are the nicotine metabolite cotinine and the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) metabolite NNAL. NNAL is particularly relevant because it is a tobacco-specific carcinogenic metabolite of a powerful lung carcinogen. When NNAL is detected in urine, there must have been exposure to tobacco products: it cannot arise from any other environmental or dietary source. Our group has established the presence of NNAL in the urine of non-smokers, essentially throughout life, and in a variety of different exposure scenarios. The results of these studies have clearly demonstrated the uptake and metabolism of a lung carcinogen by non-smokers exposed to secondhand smoke. These studies have helped to drive clean air legislation which prohibits smoking in public places in many states of the U.S., as well as abroad. This legislation, along with increased taxation and anti-tobacco advertising has helped to decrease the prevalence of cigarette smoking and lung cancer mortality in the U.S.

S 835 STRUCTURAL AND FUNCTIONAL CNS EFFECTS OF LEAD IN ADULTS.

B. S. Schwartz. *Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

The toxicity of lead exposure from many sources has been documented in increasingly more elegant and complex studies. Early epidemiologic studies focused on the impact of lead toxicity on the neurological growth and development of children, and generally relied on simple and convenient measures of dose (e.g., blood lead) and health outcomes (e.g., IQ). We have been interested in what we believe is an equally vulnerable time of life, the sixth or seventh decade, when many age-related changes are also occurring, in persons whose exposures peaked decades earlier. Our focus has been to evaluate more complex causal pathways (e.g., evaluation of gene-lead and social environment-lead interactions); to compare associations of health outcomes with biomarkers of recent (e.g., blood lead) and cumulative (i.e., tibia lead by K-shell X-ray fluorescence) dose; to incorporate a variety of outcomes, including cognitive function, structural MRI (e.g., CNS volumes and white matter lesions), and most recently, diffusion tensor imaging; and to utilize new methods in computational biostatistics. We have documented a number of novel findings regarding the structural and functional consequences of lead exposure in adults, in three populations with past or current occupational, or environmental, exposures.

Study populations at enrollment ranged from 930 to 1140 subjects; each study is longitudinal in design with between three and nine study visits per subject. The findings document that lead causes progressive declines in cognitive function over time, a form of accelerated aging in the CNS; an increase in the prevalence and severity of white matter lesions; and a loss of volume in a range of brain structures from large to small that is at least persistent, if not progressive. The research has important implications for global health and the critical goal of further reducing lead exposures worldwide, and for strategies to improve secondary and tertiary prevention of these effects in an aging world population.

S 836 VASCULAR DEVELOPMENTAL TOXICITY: IDENTIFICATION, PRIORITIZATION, AND APPLICATION.

T. B. Knudsen and N. C. Kleinstreuer. *ORD / NCCT, U.S. EPA, Research Triangle Park, NC.*

Advanced research approaches to vascular developmental toxicity, including new computational modeling methods, high-throughput in vitro tests, alternative platforms such as embryonic stem cells and zebrafish reporter gene constructs, and potential chemical prioritization, risk-assessment, and clinical applications to human health are important issues. The formation of the vascular network during embryogenesis is crucial to all aspects of development, both through maternal placental oxygen and nutrient delivery as well as direct effects on morphogenesis. Disruption in local signal-flow phenomena during development alters morphogenetic gradients that control endothelial cell differentiation, proliferation, chemotaxis, and subsequent signals. This, in turn, affects the distribution of nutrients, metabolic products and/or chemicals, and seriously impacts organogenesis. Due to the centralized role and complexity of the signaling networks underlying blood vessel formation and remodeling and their centralized role across all embryonic systems, developmental defects mediated by disruption of the vascular network can be manifested in diverse ways and attributed to a variety of factors. Associated severe developmental effects include embryonic lethality and in less severe cases, phenotypes such as preeclampsia, microphthalmia, and limb defects. Traditional prenatal animal testing is not informative for resolving and attributing the inherent phenotypic ambiguity that arises due to various vascular network perturbations, prompting a new paradigm for toxicity testing (NRC 2007). There is a need to deepen current understanding of developmental vascular biology, to identify biologically significant perturbations in potential toxicity pathways that may lead to vascular disruption, and to develop and implement new testing strategies with greater specificity and predictive power to identify the many vascular signaling pathways during developmental angiogenesis that are highly conserved and factor heavily in pathogenesis of adult vascular disease.

S 837 CELLULAR AND MOLECULAR NETWORKS UNDERLYING NORMAL AND PATHOLOGICAL EMBRYONIC VASCULAR DEVELOPMENT: INSIGHTS GAINED FROM STEM CELL RESEARCH.

K. Hirschi. *Baylor College of Medicine, Houston, TX.* Sponsor: T. Knudsen.

An understanding of vascular developmental pathology at the cellular and molecular level requires a comprehensive picture of the events leading to blood vessel formation. A key aim is to elucidate regulators of vascular cell commitment and differentiation, as well as modulators of vascular cell proliferation and migration during blood vessel assembly. We propose mechanisms by which soluble effectors, such as retinoids and TGF-α, and cell-cell gap junctions, modulate phenotype and cell cycle progression. The mouse model system is used to study the regulation of blood vessel assembly in vivo (transgenesis), in situ (embryo culture) and in vitro (coculture systems to study interactions between vascular cells and their precursors). Insights gained from the murine embryo model system are applied to modulate the commitment of human embryonic stem cells toward vascular cell fates. We are also involved in investigating the potential of adult and embryonic stem and progenitor cells to contribute to neovascularization in response to tissue injury and toxic insult. Utilizing bone marrow transplantation and localized delivery techniques, we study mechanisms by which stem and progenitor cells are recruited, induced to differentiate into vascular cells, and functionally integrated into existing vascular networks.

S 838 BIOMARKERS OF DRUG-INDUCED VASCULAR INJURY.

M. Lawton. *Pfizer, Inc., New York, NY.*

Drug-induced vascular injury (DIVI) is a toxicity that is increasingly encountered during preclinical toxicology testing. A finding of DIVI often slows or terminates development of promising drug candidates because of a lack of predictive or diagnostic biomarkers for monitoring DIVI in clinical studies. While changes in heart rate and blood pressure are easily monitored parameters known to cause DIVI for some compounds, many other drugs cause vascular damage without change in hemodynamics. Therefore, the identification of biomarkers of vascular injury is critical to advance drug development. Interestingly, many of the signaling pathways involved in embryonic vascular development are conserved throughout adulthood, and as such have high relevance to human health. Hypoxia, the production of reactive oxygen species, endothelial precursor cell activation, chemokine signaling, and many other molecular mechanisms play a role in vascular teratogenesis as well as compound-induced vascular injury in adults. The search for biomarkers of vascular injury is therefore a pursuit that will inform decisions in both developmental and acute toxicity. This presentation will focus on current efforts to identify biomarkers of DIVI and will highlight how industry and academia are working together to qualify biomarkers for use in clinical studies.

S 839 RESOLVING THE THALIDOMIDE PUZZLE: IDENTIFYING EFFECTS ON THE VASCULATURE AND DEVELOPMENTAL CONSEQUENCES.

N. Vargesson. *School of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom.* Sponsor: N. Kleinstreuer.

Thalidomide gained notoriety 50 years ago, for causing severe birth deformities to children born to mothers who took the drug early in pregnancy to relieve the symptoms of morning sickness. The upper limbs were most commonly affected, however multiple systems could also be harmed, including the kidneys, genitalia, gastrointestinal tract, ears, eyes and heart. Thalidomide has anti-angiogenic and anti-inflammatory actions. As such it is now used around the world as an anti-cancer agent, as well as a treatment for inflammatory disorders including leprosy and Crohns disease. Sadly, its misuse in Africa and South America has seen a re-emergence of thalidomide affected children.

Using chick and zebrafish embryos we recently identified thalidomide's primary teratogenic target tissue: highly angiogenic, immature blood vessels. By contrast mature, non-angiogenic vessels were temporarily stunted, but unharmed. By using analogs of the drug we confirmed only the anti-angiogenic action of the drug was responsible for teratogenesis. We demonstrated the drugs actions on immature vessels could explain the known time-sensitive actions of the drug during development and the range of defects seen.

Ongoing work is aimed at addressing several challenges. What is the molecular target of thalidomide in the endothelial cell? Can an effective form of the drug be engineered or identified that retains anti-inflammatory actions, but not the anti-angiogenic actions, therefore eradicating the potential side-effects? How does the loss of blood vessels in the developing limb result in the stereotypic thalidomide-induced limb deformity, phocomelia (where distal structures remain but proximal structures are lost in the fore and hind limbs)? The chick and zebrafish embryos are ideal for such studies, given their rapid development, ease of imaging and analysis and have provided insight into this notorious drugs mechanism of action.

S 840 COMPUTATIONAL MODELING OF EMBRYONIC VASCULAR DEVELOPMENT: AN *IN SILICO* TESTING PLATFORM FOR VDC PRIORITIZATION.

N. C. Kleinstreuer. *ORD/NCCT, U.S. EPA, Durham, NC.*

Recent studies have shown the importance of blood vessel formation during embryo development and the strong correlation to teratogenicity. Several developmental toxicants, such as thalidomide, have been identified which specifically target the forming embryonic vasculature. In an analysis of ToxCast HTS data from 467 assays and 309 high priority pesticides/chemicals, a number of developmental toxicants were found to also disrupt in vitro assays for specific targets or cellular processes important to vasculogenesis and angiogenesis. The predictive signature built from ToxCast data for several in vivo developmental endpoints from EPA's ToxRefDB database includes a pro-inflammatory/anti-angiogenic chemokine network, elements of the vascular endothelial growth factor (VEGF) signaling pathway, and the plasminogen activating system (PAS) of enzymes and growth factors mediating matrix remodeling and local signaling during blood vessel growth. We hypothesize that embryonic microvascular networks are targets for certain environmental compounds with teratogenic potential, and have identified a group of putative Vascular Disruptor Chemicals (VDCs) from the ToxCast Phase I dataset. EPA's Virtual Embryo project is building in silico models of morphogenesis to test mechanistic hypotheses by simulating developmental toxicity effects. To better characterize and prioritize chemical impact on vascular development, the systems-based model tests flow-signal interactions and predictive signatures for developmental defects associated with disturbance of morphogenetic signals, nutrients, and metabolic products. The agent-based computational model of vascular development is developed in CompuCell3D software and supplemented through semi-automatic

knowledgebase creation. Results are presented examining the teratogenic effects of alterations in the critical pathways, and simulations reveal the subsequent disruption of normal vascular network formation following modified genetic activity.

W 841 PROTEIN AGGREGATION AND NEURODEGENERATION.

G. W. Miller. *Environmental Health, Emory University, Atlanta, GA.*

Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington disease, prion diseases, and amyotrophic lateral sclerosis (ALS) afflict millions of U.S. citizens. In each of theses diseases, aggregation of various disease-specific proteins appears to play a primary role in the pathology. This process of protein aggregation now appears to be a fundamental element of disease. Recent discoveries over the past few years have greatly advanced our understanding of this important neurotoxic mechanism. Thus we will use this platform to explore the potential role of environmental factors in the protein aggregation process, potentially opening up a new avenue of research in toxicology.

W 842 PROTEIN MISFOLDING AND NEURODEGENERATION.

S. L. Ackerman. *Jackson Laboratory, HHMI, Bar Harbor, ME.* Sponsor: G. Miller.

Neuron death in age-related neurodegenerative diseases is often associated with the accumulation of misfolded proteins. Although some of these abnormally folded proteins result from mutations in the genes that encode these disease-associated proteins, little is known regarding the general mechanisms that lead to neurodegeneration associated with protein aggregation. We have taken a forward genetic approach to identify molecular pathways that when disrupted, result in the accumulation of misfolded proteins in post-mitotic neurons. To do this, we have identified the underlying molecular defect in mice with spontaneous recessive mutations that result in adult-onset neurodegeneration accompanied by the formation of protein aggregates. In addition, we have begun genetic screens to identify modifier genes that suppress protein misfolding in mutant mice.

W 843 PROTEIN AGGREGATION IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE.

M. Lee. *University of Minnesota, Minneapolis, MN.*

My group is using transgenic mouse models to study the mechanisms of human neurodegenerative diseases, particularly AD and PD. There are three broad areas of active interest. 1) Understanding the pathogenesis/progression of PD using alpha-synuclein and LRRK2 transgenic mouse models. 2) Mechanisms of amyloid-dependent neurodegeneration using transgenic mouse models of AD. 3) Pathologic interactions between genetic and environmental factors. Protein aggregation is a common pathogenic mechanism in AD and PD and we are especially interested in factors that regulate protein aggregation. While most cases of AD and PD are "sporadic", the key assumption is that genetic lesions that cause classic forms of the relevant neurodegenerative diseases will cause neural abnormalities that are common between the genetic and sporadic forms of the disease. When used in conjunction with careful analysis of human subjects, invertebrate models, and cell culture models, we will be able to define mechanisms that are directly relevant to the pathogenesis and identify possible targets for therapeutic intervention. The models generated are also essential for cross-platform screening and validation of novel therapeutic approaches. My group has identified several robust biochemical, pathological and behavioral outcome measures in transgenic mouse models of AD and PD. These measures will be essential for in vivo preclinical evaluation of therapeutics.

W 844 THE ROLE OF LIPID-PROTEIN INTERACTION IN THE PATHOGENESIS OF PRION DISEASE.

J. Ma. *Molecular and Cellular Biochemistry, Ohio State University, Columbus, OH.* Sponsor: A. Kanthasamy.

Prion diseases are a group of fatal neurodegenerative disorders that are sporadic, inherited or transmissible. We showed that Escherichia coli expressed recombinant prion protein (rPrP) binds to lipid molecules, and the lipid-rPrP interaction is significantly influenced by diseased-associated mutations and polymorphism, supporting the relevance of lipid-rPrP interaction to the pathogenesis of prion disease. Moreover, we revealed that, in the presence of lipid molecules, the soluble and protease sensitive rPrP can be converted into an aggregated and protease resistant form (rPrP-res). More importantly, the rPrP-res form can seed the conversion of rPrP molecules into the rPrP-res form, which is the key characteristic of a prion. When injected into wild-type mice, the rPrP-res causes neurodegeneration with a relatively short incubation period. The diseased mice exhibit classic manifestations of prion disease: severe spongiosis in the brain, accumulation of aggregated PrP, and the capability to serially transmit the disease. Our findings highlight the importance of lipid-PrP interaction in generating the infectious prion conformation and provide solid evidence supporting the prion hypothesis.

W 845 ROLE OF DIVALENT METALS IN PRION PROTEIN UPREGULATION AND AGGREGATION.

A. Kanthasamy, C. Choi, D. Martin, V. Anantharam, J. A. Richt and A. Kanthasamy. *Biomedical Sciences, Iowa State University, Ames, IA.*

The key biochemical event in the pathogenesis of prion diseases is the conversion of normal cellular prion proteins (PrPc) to the proteinase K (PK) resistant, abnormal form (PrPsc); however, the cellular mechanisms underlying the conversion remain enigmatic. Binding of divalent cations such as copper to the octapeptide repeat regions of PrP has been shown to be important for the stability of the protein. Recently, we demonstrated that prion protein interacts with metals and attenuates metal neurotoxicity. We also showed that cellular prion protein attenuates oxidative stress induced activation of proapoptotic events. Additionally, we examined the role of divalent transition metals (Mn2+ and Cu2+) on PrPc expression and degradation. Neuronal cells expressing mouse prion proteins with a genetically altered novel epitope (mAb 3F4) and brain slices were exposed to Mn2+ and Cu2+ over 24hr. Levels of PrPc protein and mRNA were measured. Interestingly, both Mn2+ and Cu2+ increased PrPc levels in both cytosolic and membrane-rich fractions. Notably, metal treatment neither increased PrP mRNA transcripts nor altered the mRNA stability, indicating that metals may act at the post-translation and/or degradation levels of PrPc. However, metal exposure did not alter the proteasomal or lysosomal degradation pathway. Pulse-chase analysis showed that the PrPc turnover rate was significantly decreased with metal treatment. Limited digestion with PK also revealed that metal treatment decreased the digestion rate of PrPc. These data suggest that divalent metals can alter the normal processing of PrPc, resulting in the accumulation of PrPc with altered susceptibility to PK. Additionally, exposure of Mn to an infectious prion cell model, mouse RML-infected CAD5 cells,increased prion protein levels. These data suggest that divalent metals can alter the normal processing of PrPc, resulting in the accumulation of PrPc with altered susceptibility to PK. These findings indicate a pivotal role of metals in the pathological progression of prion disease and other proteinopathies, including PD.

W 846 TECHNICAL CHARACTERIZATION AND DOSIMETRY CHALLENGES ASSOCIATED WITH CONDUCTING OR INTERPRETING NANOTOXICITY.

S. F. Ali[1] and S. Hussain[2]. [1]*Neurotoxicology, NCTR, Jefferson, AR and* [2]*HED Air Force Research Laboratory, Wright-Patterson Air Force Base, Dayton, OH.*

Recent developments in nanotechnology have generated a degree of apprehension concerning the potential risk to human health and the environment from manufactured engineered nanomaterials (MEN). The unique chemical and physical properties of MEN coupled with their high surface area per unit mass require a re-assessment of exposure protocols and models to assess the toxicity of MEN. Based on ultrafine particle toxicity knowledge and recent toxicity studies on new forms of MEN, the biological activity of MEN will depend on specific physicochemical characteristics that are not usually considered in dissolution chemical toxicological studies. Not only must the size and surface area of the MEN be characterized prior to dose but the size distribution, chemical composition, crystallinity, surface structure, shape, and solubility must be determined. Additionally, the MEN in solution may be destabilized before, during, or after dosing resulting in an agglomeration rate that is dependent on MEN preparation methods and the biological system that the MEN are introduced into. Since agglomeration can modify uptake rates, transport, and clearance by the cell model or organ system, it is critical to interpret the data from MEN toxicity experiments with a detailed knowledge of the physicochemical properties of the MEN at all experimental time points. Therefore we must consider the toxicity assessment of MEN and address technical challenges associated with conducting or interpreting in vitro or in vivo toxicity studies. We will also discuss the technical challenges associated with obtaining accurate and reproducible results and the selection of appropriate controls. Optimal methods for dispersing MEN in solution, monitoring agglomeration, and methods of tracking and visualizing MEN after internalization will be presented. Overall, this important forum will provide for the discussion of these critical issues in the newly emerging field of nanotoxicity.

W 847 ROBUST CHARACTERIZATION OF NANOMATERIALS IS NECESSARY BEFORE TOXICITY STUDIES/ASSESSMENT CAN BE UNDERTAKEN.

D. B. Warheit. *DuPont Haskell Global Centers, Newark, DE.*

Numerous scientific organizations have strongly recommended that investigators should adequately characterize physicochemical properties of the Nanoparticle-types that are being evaluated for hazard testing. This is because, in the absence of a rigorous characterization of the nanoparticle-types being studied, the published results will have limited value, significance or biological relevance. This is due to 1) the uncertainty of the specific substance identity of the test substance; and 2) the inability of others to conduct comparable or confirmatory studies with that particular nanoparticulate-type. Thus, robust characterization of nanoscale particle-types is a necessary prerequisite prior to implementing hazard assessments. However, too often this requirement results in confusion associated with a "laundry list" of numerous "required", physicochemical characteristics which may not have adequate prioritization. In an effort to obviate this relative state of confusion and to provide some form of pragmatism to this process, it is proposed that, at a minimum, toxicologist should characterize the following (prioritized) physicochemical properties prior to conducting hazard studies with nanoparticle-types: Particle size and size distribution (wet state) and surface area (dry state) in the relevant media being utilized – with strong consideration of the route of anticipated exposure; Crystal structure; Aggregation status in the relevant media; Composition/surface coatings; Surface reactivity; Method of nanomaterial synthesis and/or preparation including post-synthetic modifications; and Purity of samples. This represents a minimum, standardized assessment of physiochemical properties that should be analyzed and documented prior to the development of toxicity testing with nanoparticles. More importantly, this will permit analyses and comparisons of studies with similar or identical particulate-types and contribute to the coherence of the nanoparticle hazard database.

W 848 MINIMUM MATERIAL CHARACTERIZATIONS FOR NANOTOXICOLOGY STUDIES: A NECESSITY OR A NUISANCE?

N. J. Walker. *National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Nanoscale materials (nanomaterials, nanoparticles), are a broadly defined set of substances that have at least one critical dimension less than 100 nanometers and possess unique optical, magnetic, or electrical properties. The unique and diverse physicochemical properties of nanoscale materials suggest that toxicological properties may differ from materials of similar composition but different size. Ongoing research is showing that decreasing particle size below barrier cutoffs for portals of entry can lead to new unintended routes of exposures. In addition, once internalized, surface-based interactions can have profound impacts on the kinetics and biodistribution of materials of similar size and shape leading to large differences in target organ dosimetry. Finally, for some nanomaterials, "dose" for certain responses can scale with a size-dependent property such as surface area, thus assessments of relative risk based on mass-based dose may lead to erroneous assertions of relative risks. Because of the need to understand how the physical and chemical aspects of a given nanomaterial impact these issues, its has been suggested that a specified minimum set of nanomaterial characteristics should be required for all papers that are published on nanomaterials. However what that minimum set of characterizations should be, in what context they should be applied and whether they should be a applied or are appropriate for a specific nanomaterial are still questions under debate.

W 849 THE SELECTION AND CHARACTERIZATION OF NANOMATERIALS FOR DEVELOPING TOXICOLOGICAL QSARS.

S. Oldenburg[1], A. Neigh[1], O. Nguyen[1] and S. Ali[2]. [1]*nanoComposix, San Diego, CA and* [2]*NCTR/FDA, Jefferson, AR.*

The number and variety of nanomaterials that pose an exposure risk is rapidly increasing. Since it is not possible to perform in-depth toxicity studies on each of these emerging nanomaterials, it is important to identify the physical and chemical properties of nanoparticulates that are linked to increased toxicity in biological systems. A silver nanoparticle panel has been developed that contains over 80 different nanoparticle variants with precisely controlled size, shape, charge and surface. The particles are unagglomerated, concentrated and purified from residual reactants. By performing experiments where only a single nanoparticle characteristic is altered, toxicological quantitative structure activity relationships (QSARs) can be determined in the model biological system. Initial studies with this panel have demonstrated that both the EC50 minimum inhibition concentration for E. coli as well as cytotoxicity in RAW 264.7 cells correlates strongly with surface area across nanoparticle sizes that range from 20 to 100 nm. However, there is only a small subset of material systems where the nanoparticle characteristics can be so precisely controlled. For the other material systems, the nanoparticle properties are dictated by manufacturing methods. Strategies for generating nanoparticles sets made from less controllable material systems that are still useful for linking toxicity to physical and chemical properties will be presented.

W 850 DOSIMETRIC CONSIDERATIONS FOR EXPLORING THE CYTOTOXICITY OF SILICA NANOPARTICLES *IN VITRO.*

D. F. Lison[1], V. Rabolli[1] and L. Thomassen[2]. [1]*Louvain Centre for Toxicology and Applied Pharmacology, Brussels, Belgium and* [2]*Centrum voor Oppervlaktechemie en Katalyse, Katholieke Universiteit Leuven, Leuven, Belgium.*

There is currently a strong pressure to develop reliable methods allowing to assess the health hazards of nanomaterials. In vitro tests are essential tools for this purpose. However, the use of cell culture models poses some specific challenges when applied to nanomaterials. To address some of these issues, we used a set of amorphous silica nanoparticles (NP, 2-335 nm) as a model and examined (1) how to express the dose of NP in cytotoxicity assays, (2) the influence of the physicochemical characteristics of NP on cytotoxicity and (3) the role of NP aggregation in the cytotoxic response. Several cell lines and erythrocytes were used in these studies.
We found that, despite their limited diffusion and/or gravitational sedimentation rate in culture medium, all suspended silica NP reach the cells at the bottom of the culture well and contribute to the cytotoxic response. Using the nominal dose to design and/or report the results of in vitro studies with NP is, therefore, appropriate.
The response to these silica NP is governed by different physicochemical parameters which vary with cell type : in J774 cells, the cytotoxic activity increases with external surface area and decreases with micropore volume; in EAHY926 and 3T3 cells, the cytotoxic activity of the SNP increases with surface roughness and small diameter, and in erythrocytes, the lytic activity increases with the diameter of the NP. It is, therefore, possible to predict the in vitro cytotoxic potential of silica NP with good accuracy on the basis of their physicochemical characteristics. These determinants are, however, complex and vary with cell type.
Finally, we generated stable aggregates of these silica NP with increasing size but no change in specific surface area. We found in J774 and 3T3 cells that the degree of aggregation did not significantly modify the cytotoxic response, which is consistent with the major role of surface area in governing the cytotoxic response to insoluble NP.

W 851 ENABLING PREDICTION AND EXTRAPOLATION: A NEW PARADIGM FOR NANOMATERIAL DOSIMETRY *IN VITRO.*

J. G. Teeguarden[1], P. M. Hinderliter[2], G. Orr[3], K. R. Minard[2], B. D. Thrall[2] and J. G. Pounds[2]. [1]*Biological Monitoring and Modeling, Pacific Northwest National Laboratory, Richland, WA,* [2]*Biological Sciences, Pacific Northwest National Laboratory, Richland, WA and* [3]*Environmental and Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA.*

Current practice in in vitro nanomaterial toxicity studies is to ignore the issue of cellar dose in favor of measures of exposure (media concentration). While appealing for their simplicity and ease of calculation, use of exposure metrics such as mass media concentration (μg/ml) introduce significant errors the hazard assessment process and obstruct extrapolation of in vitro findings equivalent in vivo exposure levels for risk assessment purposes. Like particles and chemicals in vivo, nanoparticles and their larger relatives have unique kinetic processes in vitro. Particles diffuse and sediment at rates that depend on their size, density and agglomeration state, as well as the properties of the media. Thus, the dose-rate for all particles are not equal, but can vary significantly, in direct contrast to the assumption of dose-equivalency implicit in the use of mass based media concentrations as metrics of exposure for dose-response assessment. Accounting for particle dependent dosimetry either by computational kinetics or direct measurement would overcome this major barrier to effective application of in vitro studies for nanomaterials. Most importantly, replacing exposure measures with measures of target cell dose would bring the field in line with current practice for chemicals and particles and enable extrapolation of in vitro findings to in vivo exposures. This talk will present a new, flexible paradigm for nanomaterial dosimetry in vitro which is consistent with current practice for chemical risk assessment, reflects particokinetics in vitro, and can be used to extrapolate accurately between in vitro systems, across particle types and from in vitro to in vivo.

W 852 UNDERSTANDING STRUCTURAL AND PHYSICAL CHEMICAL DRIVERS OF DRUG TOXICITY: UTILITY AND TRANSLATABLE VALUE.

D. P. Hartley[1] and M. Kansy[2]. [1]*Genentech, South San Francisco, CA and* [2]*F. Hoffmann-LaRoche AG, Basel, Switzerland.*

Given the demands on drug discovery teams to produce effective, specific, and safe new molecules, toxicologists are now finding themselves with new responsibilities in the drug discovery setting as part of lead optimization teams. To be effective members of these teams, toxicologists are now required to gain awareness and experience in recognizing potential structural and physicochemical properties of compounds that often lend to certain off-target toxicities, such as off-target receptor activities, QT-interval prolongation, phospholipidosis, enzyme induction, liver injury, and genotoxicity. The understanding of structural attributes of compounds that may drive specific liabilities will enhance the toxicologist's contributions by enabling active collaboration with chemists to inform synthesis of new molecules to gain a safety advantage over previous molecules. To this end, in vitro assays (e.g., hERG channel inhibition, pharmacology ligand binding panels, enzyme induction, and transporter inhibition), coupled with specific mathematical or statistical models, and pharmacophore models (e.g., hERG channel and PXR activation) can be effective at instructing chemical modifications to attenuate the potential for activity against specific toxicological endpoints a priori. Furthermore, new in silico approaches in chemoinformatics are proving useful for predicting off-target polypharmacology by mapping potential drug-target interactions through ligand-based similarities. Similarly, mapping adverse drug reactions to chemical space may hold promise for predicting specific features of compounds that lead to adverse drug reactions in humans. Finally, detailed assessments of pharmacokinetic or physical chemical drivers of tissue accumulation are improving our understanding of in vitro—in vivo correlations. Overall these approaches demonstrate the importance and utility of understanding structural motifs and physicochemical properties of compounds that lead to off-target activity.

W 853 CLINICAL TRANSLATIONAL VALUE OF PHARMACOLOGICAL PROMISCUITY OBSERVED IN *IN VITRO* SAFETY PROFILING.

L. A. Urban[1], S. Whitebread[1], J. Hamon[2], K. Azzaoui[2] and D. Mikhailov[1]. [1]*Center for Proteomic Chemistry, NIBR, Cambridge, MA and* [2]*Center for Proteomic Chemistry, NIBR, Basel, Switzerland.*

In vitro safety profiling of a large number of pharmacological targets has recently gained significant contribution to drug discovery. Extensive panels of biochemical and functional assays addressing single targets in native or engineered cellular environment provide large volume of data. In vitro safety profiling determines whether compounds affect off-targets associated with ADRs and their pharmacological promiscuity. In silico tools made it possible to deconvolute and interpret these data and combine the chemistry and pharmacology space. Integrated assessment of data today includes ADME, metabonomics and epigenetic considerations.
We focus here on pharmacological promiscuity, an important element of early profiling. Excluding psychiatric drugs, marketed medicines show little promiscuity. Most of these drugs are safe in the clinic, while those with high hitrate show more serious ADRs and often attract black box warnings or labels.
Analysis of compounds from different phases of drug discovery show on average higher pharmacological promiscuity then marketed drugs and high attrition rates associated with safety during late preclinical development and in clinical trials show that they are more likely to fail to reach the clinic.
Pharmacological promiscuity can be associated with physicochemical properties (logP,PSA). In addition structural features of molecules can be recognized within SAR for promiscuity over a broad range of targets/target families. We will highlight association of SAR with off-target activities which could be successfully mitigated during drug discovery. In vitro-in vivo correlations, determination of PD/PK-based estimates of safety index and corresponding clinical therapeutic index should be part of drug discovery decision trees and flowcharts. These preclinical forms of evaluations place in vitro safety pharmacology data in clinical context and investigate whether a molecule within a particular structural class could satisfy requirements for a medicine.

W 854 PREDICTING DRUG OFF-TARGETS UNDERLYING ADVERSE EVENTS.

M. Keiser[1, 2] and B. Shoichet[2]. [1]*SeaChange Pharmaceuticals, Inc., San Francisco, CA and* [2]*Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA.* Sponsor: D. Hartley.

Chemically similar drugs often bind biologically diverse targets, yet most drugs are presumed selective at therapeutic concentrations. To investigate this assumption, we used the Similarity Ensemble Approach to compare several hundred approved drugs against a panel of over 170,000 ChEMBL ligands organized into thousands of sets according to the targets they modulate. Novel off-target drug activities consistent with adverse events were predicted and confirmed in pharmacological assays. In several cases, the drug's novel target may be more consistent with the adverse event than are any of its previously known targets. The chemical similarity approach used here is systematic and comprehensive, and may find use for illuminating side effects of approved drugs.

W 855 EARLY SAFETY PROFILING IN DRUG DISCOVERY: HOW STRUCTURAL AND PHYSICOCHEMICAL COMPOUND CHARACTERISTICS INFLUENCE THE SAFETY PROFILE OF DRUG CANDIDATES.

M. Kansy and H. Fischer. *Nonclinical Safety, F. Hoffmann-La Roche Ltd., Basel, Switzerland.*

The determination and optimization of drug compound characteristics in fast feedback loops is an important activity in the lead identification (LI) and lead optimization phase (LO) of drug discovery research. Usually in vitro assays are applied at these phases. With the increasing number of available safety relevant measurement results predictive (in-silico) models for safety assessments can be developed. The combined application of in silico and specific in vitro methods allows the overall reduction of wet lab experiments and the prediction of potential safety liabilities of candidate molecules before they are synthesized (virtual compounds). Thus, the influence of compound structural and property changes on the safety profile can be partly evaluated. Compounds with improved overall quality can be selected and cost as well as attrition rate be reduced. The talk will focus on newest achievements in the early assessment of safety liabilities by in silico tools with focus on phospholipidosis, phototoxicity, cardiovascular toxicities. The importance of amphiphilic vector calculations for phospholipids prediction will be emphasized. New multivariate in silico models based on UV-characteristics and compound properties will be described and several computational models for prediction of cardiovascular safety liabilities discussed.

W 856 PHARMACOKINETIC DRIVERS OF TOXICITY FOR SMALL MOLECULES: EVALUATING PLASMA-TISSUE CONCENTRATION RELATIONSHIPS.

D. Diaz. *Investigative Safety Assessment, Genentech, South San Francisco, CA.*

Plasma drug exposures are routinely used in the interpretation of animal toxicity data. In reality, target tissue exposures can be much higher than plasma exposures, which could have implications in the understanding of in vivo toxicities and in the determination of in vivo-in vitro correlations. Examples will be presented in which in vitro-in vivo correlations and differences in species sensitivity can be better understood by considering target organ exposures. Systematic analysis of tissue drug levels for a group of structurally diverse small molecules reveals large differences in tissue distribution; in addition, tissue exposure levels show improved correlation with toxicity compared to plasma exposure levels. Analysis of pharmacokinetic parameters for these compounds indicates that tissue distribution can be roughly predicted by taking into consideration the volume of distribution (Vss) and the clearance (CLp) of a particular molecule. These findings suggest that consideration of these pharmacokinetic parameters could potentially help provide more relevant and translatable safety information.

W 857 MAPPING ADVERSE DRUG REACTIONS IN CHEMICAL SPACE.

J. Scheiber. *Pharmacology Research & Early Development Informatics, Disease & Translational Informatics, Pharmacology Research and Early Development (pRED), Penzberg, Germany.* Sponsor: D. Hartley.

This talk will introduce a novel method that links substructures of drug molecules to adverse drug reactions (ADRs) on a large scale. By leveraging the content of the Pharmapendium database we were able to generate statistical models for many ADRs based on chemical substructures. For the modeling we used the well-established Scitegic fingerprints as input for a Naïve Bayesian classifier. For each of the models we extracted the most significant chemical fragments so that a map of these could be generated. Each adverse drug reaction is represented by a node and is linked to other ADRs if they share at least one significant fragment. These results are then visualized by using Cytoscape, the state-of-the-art network visualization

platform. This novel type of visualization allows not yet-seen insights and interpretations on how certain ADRs are connected in chemical space. Several examples and extensions of the method will be discussed.

PL 858 INNOVATIONS IN TOXICITY TESTING – AN INTEGRATED APPROACH TO TESTING OF A NEW AGROCHEMICAL BASED ON SOUND SCIENCE AND THE "3RS" PRINCIPLES ON ANIMAL RESEARCH.

C. Terry[1], R. J. Rasoulpour[2], B. Gollapudi[2] and R. Billington[1]. *[1]Dow AgroSciences LLC, Oxfordshire, United Kingdom and [2]The Dow Chemical Company, Midland, MI.*

The toxicology development program for a new agricultural molecule (X11422208) provided an opportunity to modify standard testing strategies and study designs by undertaking, for the first time, an integrated approach to 1) applying 3Rs (Replacement, Refinement and Reduction) principles, 2) generating toxicokinetic (TK) data across studies, and 3) including mode-of-action (MoA) end-points to facilitate early, informed decision-making.
Approaches to the 3Rs included modifying standard palatability studies to provide additional information, performing toxicokinetic analysis without using satellite animals, and obtaining MoA data from regulatory studies. For example, the 90-day rat study included many integrated components such as immunotoxicity based on OPPTS guideline 870.7800, neurotoxicity following OECD and OPPTS guidelines and tissue sampling to aid MoA investigations.
Performing TK in all toxicity studies generated comparative blood and urine TK/metabolism data across dose levels, sexes, study durations, species, strains and life stages. These data may also be useful to provide perspective on biomonitoring data where human blood levels could be compared to NOAEL/LOAEL blood concentrations from animal studies, to inform human health risk assessment and to draw meaningful animal to human correlations.
Finally, decision-making was facilitated by providing early information, including MoA data (versus retrospective investigations at the end of the program) to predict potential effects in longer-term studies (e.g., carcinogenesis), to provide clues on potential human relevance, to aid study design and dose selection (e.g., kinetically-derived maximum dose levels) and to optimize the collection of desirable information from a minimum number of animals. These innovative approaches are introducing a new paradigm in pesticide regulatory toxicology testing with primary drivers being animal welfare and sound science potentially leading to more informed human risk assessment.

PL 859 CROSS-SPECIES GENE EXPRESSION CHANGES IN PRIMARY HEPATOCYTES EXPOSED TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN.

J. Rowlands[1], R. A. Budinsky[1], A. A. Dombkowski[2] and R. S. Thomas[3]. *[1]Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, MI, [2]Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI and [3]Center for Genomic Biology and Bioinformatics, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Human and rat primary hepatocytes were used to explore species-specific differences in gene expression profiles after treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Hepatocytes from five adult women and five female Sprague-Dawley adult rats were exposed for 24 hours to eleven concentrations of TCDD spanning 7-log concentrations (0.00001 nM to 100 nM) and the mRNA expression changes measured on whole genome arrays. Comparison of the orthologous genes with a minimum 1.5 fold change revealed that only a very small number of genes were upregulated in rat and human cells with 11 genes and 12 genes affected, respectively. Of these, 7 genes were in common between rats and humans and these were largely AHR core battery genes. Pathway enrichment analysis was performed on the most positively and most negatively correlated orthologous genes (r > 0.7). For genes with positive dose response correlation, cross-species comparison showed enrichment in AHR signaling and xenobiotic metabolizing enzyme pathways. A cross-species comparison of genes with a negative dose response correlation showed enrichment in immune system pathways. Benchmark dose (BMD) analysis was conducted on AHR core genes CYP1A1, CYP1A2, CYP1B1 and NQO1 and on AHR regulated signaling pathways. The median human to rat BMD ratio was 7.1 for individual genes and 6.5 for pathways. On both a gene- and pathway-basis, the results indicate significant differences exist in the response of rats vs. humans to TCDD and the data support cross-species adjustment factor of 1.0 or less for risk assessment of TCDD.

PL 860 COMPARATIVE QSAR STUDIES OF MONO-HYDROXYLATED POLYCHLORINATED BIPHENYLS AND THEIR POTENTIAL ESTROGENIC EFFECTS.

P. Ruiz, O. Faroon, B. Fowler and M. Mumtaz. *Department of Toxicology and Environmental Medicine, ATSDR, Atlanta, GA.*

Mono-hydroxylated Polychlorinated Biphenyls (OH-PCBs) represent a new health and environmental concern because they have been shown to have agonist or antagonist interactions with hormone receptors (HRs) or hormone-receptor mediated responses. Preliminary comparative Quantitative Structure Activity Relationship (QSAR) analyses have been performed with 71OH-PCBs for estrogenic activity (human estrogen receptor ERalpha) using two-dimensional (topological and structural) and three-dimensional (spatial) descriptors. The chemometric tools used for the analyses are stepwise multiple linear regression, partial least squares, and recursive partitioning. The data set was divided into a training set and test set, and models were developed from the training set compounds. The best model was selected based on the highest external predictive q2 (cross-validation) value (0.932) and the lowest standard error of prediction value (0.64). The developed QSAR equations suggest the importance of the position of hydroxyl substitution on the phenyl ring, polarizability and the contribution of adjacent unsubstituted carbon pairs towards greater activity of these chemicals. When fully developed this model may be used for prediction of toxicity and for identification of potential adverse health consequences of untested OH-PCBs and serve as a useful tool for the safety and risk assessment of these chemicals. Further development of the model and evaluation of findings could enable mechanistic insight into the estrogenicity effects of OH-PCBs.

PL 861 MOLECULAR FIELD TOPOLOGY ANALYSIS OF STRUCTURAL DETERMINANTS FOR ACUTE AND DELAYED NEUROTOXICITY OF O-PHOSPHORYLATED OXIMES.

G. F. Makhaeva[1], E. V. Radchenko[2], V. B. Sokolov[1], V. A. Palyulin[2], N. S. Zefirov[1, 2] and R. J. Richardson[3]. *[1]Institute of Physiologically Active Compounds, Russian Academy of Sciences, Chernogolovka, Russian Federation, [2]Chemistry Department, M.V. Lomonosov Moscow State University, Moscow, Russian Federation and [3]Toxicology Program, Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

We used Molecular Field Topology Analysis (MFTA), a 2D QSAR approach based on local molecular properties (e.g., atomic charges, group van der Waals radii and group lipophilicities), to create QSAR models for anti-acetylcholinesterase (AChE) and anti-neuropathy target esterase (NTE) activity and selectivity for a series of *O*-phosphorylated oximes. MFTA produces a molecular supergraph providing a common frame of reference for the set of structures under study, partial least squares (PLS)-based regression models, and graphical maps summarizing the influence of local properties on activity or selectivity. Data on inhibitor activity were represented as $\log k_i$(AChE)and $\log k_i$(NTE) [k_i, $M^{-1}min^{-1}$]; and selectivity for NTE vs AChE as $\log[k_i(NTE)/k_i(AChE)]$. Three models were obtained: (1) $\log k_i$(AChE): N=58, N_F=6, R^2=0.91, $RMSE$=0.33, Q^2=0.73, $RMSEcv$=0.58; (2) $\log k_i$(NTE): N=35, N_F=6, R^2=0.96, $RMSE$=0.26, Q^2=0.82, $RMSEcv$=0.55; and (3) $\log[k_i(NTE)/k_i(AChE)]$: N=35, N_F=2, R^2=0.80, $RMSE$=0.61, Q^2=0.70, $RMSEcv$=0.77, where N = number of compounds; N_F = number of factors in the PLS model; R^2 = squared correlation coefficient; $RMSE$ = root-mean-square error; Q^2 = cross-validation parameter; and $RMSEcv$ = root-mean-square error for cross validation. In addition to the predictive PLS regression models, maps of local descriptor influence on the anti-AChE and anti-NTE activity of the compounds as well as on the selectivity of compounds to NTE vs AChE (neuropathicity maps) were obtained. The predictive value of the models for acute and delayed neurotoxicity remains to be validated by experimental tests. Supported by ISTC project #3130 and RAS Program "Biomolecular & Medicinal Chemistry".

PL 862 FRAMEWORK FOR INTEGRATION OF HUMAN AND ANIMAL DATA FOR RISK ASSESSMENT PURPOSES: CHLORPYRIFOS NEUROBEHAVIORAL DATA AS CASE-STUDY.

A. A. Li[1], K. Lowe[1], L. McIntosh[1] and P. Mink[2]. *[1]Health Sciences, Exponent Inc., San Francisco, CA and [2]Epidemiology, Emory University, Atlanta, GA.*

Both analytical epidemiology and animal research data from the published literature are important for a comprehensive assessment of risks due to pesticide exposure to humans. Currently, risk assessments (RA) for food-use pesticides are based primarily on guideline animal toxicity studies required for registration. Approaches

for integrating epidemiology and animal results from the published literature have become increasingly important to regulatory agencies as evidenced by recently proposed EPA draft guidance and science advisory panel reviews in 2010 on this subject. Of special importance are evaluations of developmental neurobehavioral data for children's health RA. Chlorpyrifos (CPF) is used as a case study to illustrate a framework for systematic and transparent review of published neurobehavioral literature for human health RA. Steps include (1)identify human pesticide uses and exposure patterns so that relevance of duration,route and dose levels in animal studies can be determined;(2)evaluate exposure measures used in human studies relative to what is understood about mode of action, metabolism and pharmacokinetics;(3)define criteria for inclusion and utility of studies for RA;(4)tabulate both presence and absence of all findings, direction of change, key methods and exposure period from human and animal studies;(5)evaluate historical control data to assess reliability of methods and variability of control behavior especially when there is limited dose response data from one lab;(6)evaluate patterns of associations (or lack of associations) within and across different studies with respect to the following guideposts: consistency, dose-response, strength of association, temporality (taking into consideration frequency, intensity, timing and duration of exposure), as well as hypothesized modes of action. Based on this approach, we conclude that quantitative RA based on cholinesterase inhibition are adequately protective of developmental neurobehavioral effects reported in the literature for CPF.

PL 863 DEVELOPMENT OF SURFACE CLEAN UP LEVELS FOR ACUTE AND CHRONIC EXPOSURE TO TARGET CHEMICALS.

J. M. Martinez[1], S. A. Hines[2] and D. J. Chappie[2]. [1]*Office of Emergency Management, U.S. EPA, Cincinnati, OH and* [2]*Battelle, Cincinnati, OH.*

Solid surface contamination, such as that resulting from indoor pesticide applications or residual contamination from releases of chemical weapons, is increasing recognized as a knowledge gap in the development of suitable decontamination strategies and standards to ensure appropriate clean-up. This is especially important when planning for reoccupation of a public facility contaminated with toxic agents that may persist in the environment for various lengths of time. Contaminated surfaces pose a challenge for assessment due to the lack of generally accepted dermal exposure methodologies as well as appropriate techniques to integrate surface wipe measurements with exposure point concentrations. Currently, there are no formal risk-based screening levels for chemical weapons or pesticides to determine if results from surface samples or wipe samples confirm the solid surfaces were successfully decontaminated or need to be re-evaluated before reoccupation. To develop surface clean up screening levels, we performed a careful evaluation of all surface contamination assessment methodologies proposed for the World Trade Center and home methamphetamine lab clean ups. Based on careful analysis of these methods and the chemical properties of toxicants, we present putative surface wipe screening levels for both chronic and acute exposures for a list of target chemicals.

PL 864 USING PBPK/PD MODELS TO INCORPORATE CHEMICAL AND NON-CHEMICAL STRESSORS INTO CUMULATIVE RISK ASSESSMENT: A CASE STUDY OF PESTICIDE EXPOSURES.

S. C. Wason[1, 2], T. J. Smith[1], M. J. Perry[1] and J. I. Levy[1]. [1]*Environmental Health, Harvard School of Public Health, Boston, MA and* [2]*Gradient, Cambridge, MA.*
Sponsor: A. Lewis.

Cumulative risk assessment has been proposed as an approach to evaluate health risks associated with simultaneous exposure to multiple chemical and non-chemical stressors. Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models can allow for inclusion and evaluation of multiple stressors, including non-chemical, but studies have not leveraged PBPK/PD models to jointly consider these disparate exposures in a cumulative risk context. In this study, we focused on exposures to organophosphate (OP) pesticides for children in urban low-income environments, where these children would be simultaneously exposed to other pesticides (e.g. pyrethroids) and non-chemical stressors that may modify the effects of these exposures (e.g. diet). We developed a methodological framework to evaluate chemical and non-chemical stressor impacts on OPs, utilizing an existing PBPK/PD model for chlorpyrifos. We evaluated population-specific stressors that would influence OP doses or acetylcholinesterase (AChE) inhibition, the relevant PD outcome. We incorporated the impact of simultaneous exposure to pyrethroids and dietary factors on OP dose through the compartments of metabolism and PD outcome within the model, and simulated combinations of stressors across multiple exposure ranges and potential body weights. Our analyses demonstrated that both chemical and non-chemical stressors can influence the health implications of OP exposures, with up to 5-fold variability in AChE inhibition across combinations of stressor values for a given OP dose. We demonstrate an approach for modeling OP risks in the presence of other population-specific environmental stressors, providing insight about co-exposures and variability factors that most affect OP health risks and contribute to children's cumulative health risk from pesticides. This framework can be used to inform cumulative risk assessment for any compound affected by chemical and non-chemical stressors through metabolism or PD outcomes.

PL 865 ASSESSMENT OF AGGREGATE PERINATAL EXPOSURE TO BPA.

D. A. Sarigiannis[1, 2] and S. Karakitsios[1]. [1]*Institute for Health and Consumer Protection, European Commission - Joint Research Centre, Ispra, Varese, Italy and* [2]*Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki, Greece.*
Sponsor: L. Birnbaum.

The pharmacokinetic behavior of Bisphenol A, especially in the perinatal period is subject to large controversy. Recent epidemiological findings regarding inhaled BPA revealed that even at low levels of overall daily intake (0.5 μg/kg_bw) male sexual dysfunction was observed. This study aims to investigate (a) the exposure to BPA of the developing fetus during the perinatal period and reduce the uncertainty regarding internal dose determinants (fetus sulfation and BPA_Glu deconjugation by β-glucuronidase in the placenta; and (b) the effect of administration route on BPA bioavailability. For this purpose, a dynamic lifetime PBTK model was developed, capable of estimating the BPA biologically effective dose throughout several developmental stages for different administration routes. The model integrates steady state and dynamic Markov chain Monte Carlo simulations for uncertainty assessment. The biologically effective dose of the fetus during gestation is linked to the levels of BPA in the mother blood due to its high lipophilicity and the relatively small molecular weight. Fetal BED was calculated to be only slightly lower than the one of the mother. Sensitivity analysis showed that this parameter is determining for placental concentration and affects the overall fetal exposure with a sensitivity coefficient of 0.67, much higher than the contribution of the fetal intrinsic clearance (sensitivity coefficient of 0.4), confirming thus the importance of de-conjugation. BPA bioavailability from inhalation exposure in adults is significantly higher than when BPA is orally administrated (6 times higher for the same intake rate) due to lack of first pass metabolism prior to systemic circulation entrance. Exposure to 5 μg/m3 of BPA in the air corresponds to a steady state concentration in plasma equal to 0.22 μg/L. In neonates, whose overall clearance capability is limited to almost 20% of adults, differences in BPA bioavailability for different administration routes are limited to 50%.

PL 866 USE OF A PBPK/PD MODEL TO DERIVE AGE-SPECIFIC INTERSPECIES (UF_A) AND INTERINDIVIDUAL (UF_H) UNCERTAINTY FACTORS FOR CHLORPYRIFOS.

P. S. Price[1], P. M. Hinderliter[2] and T. S. Poet[2]. [1]*Toxicology & Environmental Research & Consulting, The Dow Chemical Company, Midland, MI and* [2]*Battelle Pacific Northwest Division, Richland, WA.*

Risk assessments of chemicals use uncertainty factors for establishing safe levels of exposure. The default values for these factors do not necessarily reflect current knowledge of a chemical's mechanism of action or our growing ability to quantitatively predict internal doses and responses in humans using physiologically based pharmacokinetic/dynamic (PBPK/PD) models. A PBPK/PD model of variability in response (RBC cholinesterase inhibition) to chlorpyrifos exposure in two age groups in humans (adults and three year olds) was developed. The model was used to derive complete values (including both kinetic and dynamic components) for the interspecies (UF_A) and interindividual (UF_H) uncertainty factors. The approach used to determine UF_A is to design simulations of human populations that predict the dose levels in humans that are directly comparable to the animal BMD or BMDL. The approach used for UF_H is to directly model the variation in human response, at relevant dose levels and regimens, and use the model results to determine the size of the interindividual uncertainty factor necessary to address the variation that occurs across different fractions of the population. Finally, data from age-appropriate animal studies and age-specific models of human sensitivity are used to derive separate values of UF_A and UF_H for each age group. These age-specific values avoid the need for extrapolating from adults to children. Values of UF_A and UF_H were developed for cholinergic responses to chlorpyrifos based on a $BMDL_{10}$ determined in a recent acute oral study in rats. Values of UF_A were calculated to be <2 for the adults and three year olds. Values of UF_H were found to be <2 for the two groups when 95% of the population variation in sensitivity is considered and <3 when 99% of the variation is considered. Future work will look at other age groups (infants). This work demonstrates how PBPK/PD modeling can inform and support the risk assessment process.

PL 867 ASSESSMENT OF 16 CHEMICALS ON PROLIFERATION AND APOPTOSIS IN HUMAN NEUROPROGENITOR CELLS USING HIGH-CONTENT IMAGE ANALYSIS (HCA).

M. E. Culbreth, J. A. Harrill, T. M. Freudenrich, W. R. Mundy and T. J. Shafer. *Integrated Systems Toxicology, U.S. EPA, Research Triangle Park, NC.*

The need for efficient methods of screening chemicals for the potential to cause developmental neurotoxicity is paramount. We previously described optimization of an HCA assay for proliferation and apoptosis in ReNcell CX cells (ReN), identifying appropriate controls. Utility of the assay was examined using 16 chemicals: Cd, chlorpyrifos oxon (CPO), dexamethasone, dieldrin, ketamine, Pb, maneb, methylmercury (MeHg), nicotine, trans-retinoic acid (tRA), and trimethyltin (TMT) reported to be developmentally neurotoxic and to affect proliferation and apoptosis; Dimethyl phthalate, glyphosate, omeprazole, saccharin, and sorbitol not reported to be developmentally neurotoxic or affect proliferation and apoptosis below 30μM. Cells in 96-well plates were exposed (0.001-100μM, N=3) for 24h, and proliferation and apoptosis assessed using HCA based on BrdU incorporation and activation of p53 and caspase 3, respectively. Cell viability was measured using Cell Titer-GLO(TM). None of the 16 chemicals significantly activated p53 or caspase 3 compared to actinomycin d, which increased these by ~1000%. Thus, whether these chemicals cause apoptosis in ReN cells through other pathways needs to be assessed. Compared to control, Cd, Pb, MeHg, TMT and tRA decreased BrdU by 50-80%; CPO, dieldrin, maneb, and nicotine decreased BrdU by 30-50%; dexamethasone, ketamine, dimethyl phthalate, glyphosate, omeprazole, saccharin, and sorbitol decreased BrdU by 10-30%. At the highest concentration tested, Cd, Pb, MeHg, TMT, and tRA decreased viability by >50%, while dieldrin decreased by it by ~30%; all other chemicals had no effect on viability. Effects on proliferation were always observed at lower concentrations than effects on viability. The results show that BrdU incorporation is sensitive to chemical effects on neuroprogenitor cell proliferation. Supported by EPA and the 2009 Colgate-Palmolive award. *This abstract does not reflect Agency Policy.*

PL 868 TOXIC ALTERATIONS OF DOPAMINERGIC NEURON DEVELOPMENT FROM PLURIPOTENT STEM CELLS AFTER CHRONIC LOW-DOSE EXPOSURE TO METALS DURING THE LATE MATURATION PHASE.

M. Leist, S. Schildknecht, S. Kadereit and B. Zimmer. *in vitro toxicology and biomedicine, University Konstanz, Konstanz, Germany.*

In vitro neural differentiation of embryonic stem cells (ESC) has been shown to recapitulate in vivo development of neurons. Here, murine ESCs were differentiated to neurons under feeder-free conditions. Genome-wide transcriptional profiling identified waves of gene expression corresponding to steps in neurodevelopment. Based on these findings, different windows of sensitivity to chemicals were identified. The late period of maturation of already post-mitotic neurons appeared particularly interesting, as it might corresponds to the in vivo perinatal period which is particularly relevant for developmental neurotoxicology. In a first proof-of-concept experiment the cultures were exposed to 1 μM lead acetate for 6 days during this period. Although no neurotoxicity was observed, the regulation of several disease-relevant neuronal genes was significantly affected. When we tested methylmercury (MeHg), robust effects on gene regulation were observed in the sub-nanomolar. Profiling of altered receptors and markers suggested that the subpopulation of dopaminergic neurons was affected. This form of toxicity was confirmed by functional assays measuring the activity of the dopamine transporter. The toxic signalling involved activation of the c-jun N-terminal kinase pathway, and respective inhibitors attenuated the MeHg toxicity on dopaminergic neurons. To our knowledge this test system has an unprecedented sensitivity and can be used both for mechanistic studies on known compounds and for the screening of the potential hazard of unknown compounds.

PL 869 IS THE PENTABDE REPLACEMENT, TRIS (1, 3-DICHLORO-2-PROPYL) PHOSPHATE (TDCPP), A DEVELOPMENTAL NEUROTOXICANT? STUDIES IN PC12 CELLS.

L. Dishaw[1], C. M. Powers[2], I. T. Ryde[1], S. C. Roberts[1], F. J. Seidler[2], T. A. Slotkin[2] and H. M. Stapleton[1]. *[1]Nicholas School of the Environment, Duke University, Durham, NC and [2]Pharmacology and Cancer Biology, Duke University, Durham, NC.*

Chlorinated organophosphate flame retardants (OPFRs) are expected to be a primary replacement for the commercial PentaBDE mixture that was phased out in 2004. Structurally, OPFRs are similar to the neurotoxic organophosphate pesticides, raising concerns about their toxicity. Additionally, OPFRs are persistent in the environment and have been detected at high concentrations in household dust, indicating that widespread human exposure is likely.
We compared the developmental neurotoxicity of tris (1,3-dichloro-2-propyl) phosphate (TDCPP) to the organophosphate pesticide, chlorpyrifos (CPF), a known neurotoxicant. We also compared TDCPP to three structurally similar OPFRs, tris (2-chloroethyl) phosphate (TCEP), tris (1-chloropropyl) phosphate (TCPP), and tris (1,3-dibromopropyl) phosphate (TDBPP), and 2,2′,4,4′-tetrabromodiphenyl ether, a major PentaBDE component. Using undifferentiated and differentiating PC12 cultures, we assessed changes in DNA synthesis, oxidative stress, cell viability (TDCPP only), differentiation into dopaminergic or cholinergic neurophenotypes (all OPFRs), cell number, cell growth and neurite growth.
TDCPP elicited concentration-dependent neurotoxicity, with effects equivalent to or greater than an equimolar concentration of CPF. TDCPP inhibited DNA synthesis and elevated oxidative stress without adversely affecting cell viability or growth. All OPFRs decreased cell number, and altered neurodifferentiation. TDCPP and TDBPP promoted differentiation into both neuronal phenotypes, while TCEP and TCPP promoted only the cholinergic phenotype. BDE-47 had no effect across all measures tested. These data suggest that OPFRs affect neurodevelopment with similar or greater potency as known neurotoxicants. OPFRs showed divergent patterns of differentiation, suggesting the participation of multiple mechanisms of toxicity that appear to be driven by structural differences (Support: NIH R01-ES016099 & P30-ES011961).

PL 870 MULTIPARAMETRIC LIVE CELL IMAGING OF IMPAIRED NEURITE OUTGROWTH IN HIGH-DENSITY CULTURES OF HUMAN NEURONAL PRECURSORS.

M. Daneshian, N. Stiegler, F. Matt and M. Leist. *in vitro toxicology and biomedicine, University Konstanz, Konstanz, Germany.*

Models for the outgrowth of neurites and methods to quantify modifications of this process are at the centre of interest in the field of developmental neurotoxicology (DNT). We developed an imaging-based procedure to quantify neurite growth in LUHMES human neuronal precursor cells that spontaneously extend projections after plating. Labelling methods were all based on live cell stains, to avoid additional manipulations that might affect fragile neurite structures. Although cells were grown at high density to allow extensive networking, the observer-independent test allowed for a signal-noise ratio of > 50 with regards to the quantification of overall neurite growth. General cytotoxicity was measured simultaneously with neurite evaluation for each individual cell by calcein uptake. Toxicity affecting the extension of processes occurred for compounds such as U0126 independent of general cytotoxic effects. Under such conditions, high numbers of viable cells without neurites were detected. A test set of known negative and positive controls, including also the extension-prolonging compound Y-27632 was identified correctly. This unique multi-parametric imaging approach was finally used to examine to which extent unspecific cytotoxicants such as menadione, cadmium and sodium dodecyl sulfate would affect neurites. In some cases, apparently specific disintegration of cellular extensions was observed as indirect activity of such compounds. These data suggested that only a concentration ratio of > 4 between EC50(neurites) and EC50(cell death) defines a directly neuritotoxic compound. The characteristics of the described novel test system suggest its usefulness both for high throughput screens (HTS) and for mechanistic research.

PL 871 STUDIES OF THE VARIABLES AFFECTING BEHAVIOR OF LARVAL ZEBRAFISH FOR DEVELOPMENTAL NEUROTOXICITY SCREENING.

S. Padilla[1], D. L. Hunter[1], B. Padnos[1], S. Frady[3] and R. C. MacPhail[2]. *[1]ISTD, U.S. EPA, Res. Tri. Pk., NC, [2]TAD, U.S. EPA, Res. Tri. Pk., NC and [3]NCCT, U.S. EPA, Res. Tri. Pk., NC.*

The U.S. Environmental Protection Agency is evaluating methods to screen and prioritize large numbers of chemicals for developmental toxicity. We are exploring methods to detect developmentally neurotoxic chemicals using zebrafish (*Danio rerio*) behavior at 6 days of age. The behavioral paradigm simultaneously tests individual larval zebrafish under both light and dark conditions in a 96-well plate using a video tracking system. By controlling the duration and intensity of light, we are able to assess visual threshold, changes in locomotion during light-dark transitions, and adaptation to both light and dark during approximately 1.5 hours of testing. The testing format allows evaluation of large numbers of larvae, chemicals and chemical concentrations. We have found that many developmentally neurotoxic compounds (*e.g.*, valproate, *d*-amphetamine) perturb behavior, while many developmentally non-neurotoxic compounds (*e.g.*, acetaminophen, saccharin) do not. Additionally, we have found that many variables affect the level or pattern of loco-

motor activity, including age of the larvae, size of the well, time of day that behavior is tested, and the presence of malformations. Some other variables, however, do not appear to affect larval behavior, including type of rearing solution (10% Hank's vs. 1:3 Danieau vs 60 mg/kg Instant Ocean vs 1x or 0.1x EPA Moderately Hard Water). Zebrafish larval behavior using a microtiter plate format may be an ideal endpoint for screening developmentally neurotoxic chemicals, but it is imperative that many test variables be carefully specified and controlled. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

PL 872 DRUGS THAT TARGET DOPAMINE RECEPTORS: CHANGES IN LOCOMOTOR ACTIVITY IN LARVAL ZEBRAFISH.

T. D. Irons[2, 1], P. Kelly[3], D. L. Hunter[1], R. C. MacPhail[1] and S. Padilla[1]. *[1]U.S. EPA, Research Triangle Park, NC, [2]Curriculum in Toxicology, University of North Carolina Chapel Hill, Chapel Hill, NC and [3]Biology Department, University of North Carolina Chapel Hill, Chapel Hill, NC.*

As part of an effort at the US Environmental Protection Agency to develop a rapid *in vivo* screen for prioritization of toxic chemicals, we have begun to characterize the locomotor activity of zebrafish (*Danio rerio*) larvae. This includes assessing the acute effects of drugs known to act on the central nervous system, specifically the dopaminergic nervous system. Zebrafish larvae (6 days post-fertilization) were maintained in 96-well microtiter plates (1 larva/well) at 26°C and under a 14:10 light:dark cycle with lights on at 0830 hr. Non-lethal concentrations of both selective (*e.g.* quinpirole and haloperidol) and non-selective (*e.g.* apomorphine and butaclamol) dopaminergic agonists and antagonists were administered by immersion. Each drug was screened on individual plates, with a broad range of concentrations and vehicle controls included. An initial experiment identified the time of peak effect of each drug (20-260 minutes post-dosing, depending on the drug). Locomotor activity was then assessed for a period of 70 minutes in light and dark conditions at the time of peak effect to analyze concentration-dependent effects of the drug. All of the drugs tested were found to alter the locomotor activity of 6-day old zebrafish in a dose-dependent manner. The selective agonists and antagonists caused opposing effects on activity. The dose-response profiles of the nonselective drugs were more complex, displaying biphasic patterns. This test has proven to be sensitive for detecting differing patterns of acute behavioral effects produced by drugs that act on the dopaminergic nervous system. *T.D. Irons is supported by the NIDA National research Service Award (T32 DA007244), the NIH NIGMS Initiative for Maximizing Student Diversity, and the NIEHS National Research Service Award (T32 ES007126).* *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

PL 873 EMBRYONIC EXPOSURE TO DELTAMETHRIN RESULTS IN DOPAMINERGIC GENE EXPRESSINON CHANGES AND ALTERED SWIM ACTIVITY IN THE ZEBRAFISH.

T. S. Kung, J. R. Richardson, K. R. Cooper and L. A. White. *EOHSI, Rutgers University/UMDNJ, New Brunswick, NJ.*

Pyrethroids are one of the most commonly used insecticides and, when used properly, are generally considered to pose little risk to human health. However, there has been increasing concern that children are more susceptible to the adverse affects of pesticides. Previous work from our laboratory characterized the acute developmental toxicity of six pyrethroids in zebrafish and it was determined that the toxicities associated with acute exposures are similar to what has been shown in mammalian models, thus validating the zebrafish model system. We are using the zebrafish model to test the hypothesis that developmental exposure to low doses of pyrethroid pesticides alters dopaminergic neuronal development resulting in long-term gene expression changes and behavioral alterations. To assess the effects of low dose pyrethroid exposure during development, we exposed zebrafish embryos to deltamethrin (DM) at a dose below the LOAEL (0.5 μg/L), during the embryonic period (3-72hpf) using a static non-renewal water exposure. We found that embryos exposed to DM exhibited dopaminergic gene expression changes at 72hpf, including increased expression of the dopamine transporter (DAT) (1.6-fold) and the dopamine receptor D1 (DRD1) (2.1-fold), which persists for at least 1-month post-exposure. To determine if developmental DM exposure altered behavior, we quantified the swim behavior of 2-week old zebrafish using the Noldus Ethovision System and found that treated zebrafish exhibited statistically significant increases in swim activity (3-fold), measured by the total distance traveled in 30min. Fish exhibiting altered behavior and the respective control fish were grown to sexual maturity and bred within treatment group to obtain F1 generation fish. The F1 generation exhibited decreased expression of DAT (1.6-fold) and DRD1 (2.6-fold) and increased swim activity. Our data suggest that developmental exposure to DM results in persistent dopaminergic gene expression changes and behavioral deficits.

NIEHS R56ES018863, ES005022, and ES015991

PL 874 DYSREGULATION OF HEPATIC MICRORNA PROFILES ARE ASSOCIATED WITH EARLY STAGES OF CHEMICAL CARCINOGENESIS.

C. Koufaris[1], J. Wright[2], R. A. Currie[2] and N. J. Gooderham[1]. *[1]Biomolecular Medicine, Imperial College London, London, United Kingdom and [2]Syngenta, Bracknell, United Kingdom.* Sponsor: R. Peffer.

The lack of short-term tests that predict non-genotoxic carcinogens means that their carcinogenicity is tested in rodent lifetime bioassays that are laborious, expensive, slow and prone to artifacts due to the extreme dose levels that are sometimes tested. MicroRNAs (miRNAs), non-coding RNA genes, have been implicated in carcinogenesis. Thus, changes in miRNA expression could be an early indicator of carcinogenicity. To evaluate this we used an Agilent microarray platform containing 350 rat miRNA probes to assess expression of miRNAs in the livers of male Fischer rats exposed to the maximum tolerated dose of six carcinogens and two non-carcinogens for a period of 90 days. The chemicals were chosen to represent diverse mechanisms of action (MOA) and were: 2-acetylaminofluorene (2-AAF), methapyrelene HCL (MP), chlorendic acid (CA.), monuron (MON), diethylhexipthalate (DEHP), and phenobarbital (PB). 2-AAF is a genotoxic carcinogen, MP is cytotoxic, DEHP is a PPARa agonist, and PB is a CAR activator. The MOA is not known for MON or CA. Of the two non-carcinogens benzophenone (BP) is a PB-like compound, while diethylthiourea (DETU) has few effects on the liver. One-way ANOVA identified 70 miRNAs as being differentially expressed across treatments. Hierarchical clustering showed that miRNA profiles reflect MOA. Some miRNAs were altered by several carcinogens, including two (miR-34a and miR-96) that have been identified as being continually dysregulated from normal liver to cirrhotic and then to tumor. Expression of miR-34a was induced by the three most potent carcinogens (2-AAF, MP, CA) as well as carcinogenic MON. MiR-96 was induced by PB, DEHP, MON, as well as non-carcinogenic BP. This study has shown that a range of hepatocarcinogens that differ in their MOA, all alter the expression of liver miRNAs. We identified a set of miRNAs whose expression is dysregulated at early stages of chemical hepatocarcinogenesis. Confirmation of the specificity and sensitivity of the identified miRNAs as early biomarkers of carcinogenicity is warranted.

PL 875 A LIPIDOMIC INVESTIGATION OF EARLY METABOLIC CHANGES CAUSED BY NON-GENOTOXIC CARCINOGENS.

Z. Ament[1], R. A. Currie[2], J. Wright[2] and J. L. Griffin[1]. *[1]Department of Biochemistry and Cambridge Systems Biology Centre, University of Cambridge, Cambridge, United Kingdom and [2]Syngenta, Bracknell, United Kingdom.* Sponsor: R. Peffer.

The prediction of non-genotoxic carcinogens (NGCs) has been an area of research for over half a century and still, these compounds are only identified by lengthy in vivo studies. Any advance in understanding how NGCs cause changes leading to pathological events could have impact in the development of new toxicological screening tools. A number of NGCs increase the proliferation of sub-cellular organelles such as endoplasmic reticulum and peroxisomes. In addition many NGCs have a profound impact on metabolism influencing lipoprotein metabolism, long-chain fatty acid oxidation, and lipogenesis. These changes suggest that the lipidome, the cellular compliment of lipid species may be highly sensitive to some of the earliest metabolic changes associated with initial exposure to NGCs. The present study was undertaken to understand the impact NGCs have on the lipidome of liver tissue. In this study ten compounds were chosen to cover a range of biological effects in the liver. These were phenobarbital, chlorendic acid, diethylhexyl phthalate, monuron and methapyrilene HCl which are NGCs, cinnamyl anthranilate, diethylhexyl adipate, benzophenone and Diethylthiourea, non-mutagenic non-hepatocarcinogens and 2-acetyl aminofluorene a genotoxic hepatocarcinogen. Compounds were dosed into male F344 rats, the liver was removed and lipid extracts prepared for analysis by Gas Chromtaography-Mass Spectrometry and Liquid Chromatography-Mass Spectrometry to profile changes in total fatty acids, free fatty acids, mono- di- triacyl-glycerides and phospholipids.This approach demonstrated large differences between compounds with different modes of action, and in particularly readily distinguished PPAR-α agonists, regardless of whether they are considered to be NGCs. However, multivariate analysis identified an increase in arachidonic acid containing species in rats dosed with NGCs. This suggests that there may be a common lipidomic profile associated with NGCs.

PL 876 DEVELOPMENT AND EVALUATION OF A GENOMIC SIGNATURE FOR THE PREDICTION OF NON-GENOTOXIC HEPATOCARCINOGENS IN THE RAT.

R. T. Dunn[1], A. Adai[2], A. Olaharski[3], G. H. Searfoss[4], J. Sina[5], J. Aubrecht[6], E. Boitier[7], P. Nioi[1], D. Jacobson Kram[8], N. Raghavan[9], B. Car[10], S. Chen[10], Y. Yang[11], A. Kinkaid[12], J. Sherlock[12], S. Auerbach[13] and M. Fielden[1]. *[1]Amgen, Thousand Oaks, CA, [2]Asuragen, Inc., Austin, TX, [3]F. Hoffman-LaRoche, Nutley, NJ, [4]Lilly Research Laboratories, Indianapolis, IN, [5]Merck, West Point, PA, [6]Pfizer, Groton, CT, [7]sanofi aventis, Vitry-Sur-Seine Cedex, France, [8]U.S. FDA, Silver Spring, MD, [9]J&J PRD, Raritan, NJ, [10]BMS, Princeton, NJ, [11]Abbott, Abbott Park, IL, [12]Life Technologies, Foster City, CA and [13]NIEHS-NTP, Research Triangle Park, NC.*

Evaluating risk of chemical carcinogenesis is challenging due to the protracted nature of the pathology and the translatability of animal models. The Predictive Safety Testing Consortium of the Critical Path Institute facilitates early risk assessment of new chemical entities by advancing prediction and mechanistic insight into rodent non-genotoxic hepatocarcinogenity. Extending upon prior microarray studies, we investigated a higher-throughput q-PCR signature to predict the potential for non-genotoxic compounds to induce liver tumors in rat. Using rat liver RNA from a training set of 72 compounds, a predictive model was developed on the TaqMan® Array using only 23 genes, thus providing an economical and standardized assay protocol. Testing on over 900 diverse rat liver RNA samples confirmed the inter-laboratory reproducibility of the assay and its ability to classify known non-genotoxic hepatocarcinogens. Based on variables inherent to the model and empirical data, we found that repeat dosing and the use of male S-D rats to be important for optimal classification. When tested under these conditions, signature sensitivity and specificity was 71% and 65%, respectively. Exploratory evaluations showed that prediction of carcinogenicity in other tissues based on liver gene expression is unlikely with the current model; however different modes of action for non-genotoxic hepatocarcinogens can be discriminated based on the expression of specific genes. These results define an early preclinical testing paradigm that enables a proactive risk assessment for non-genotoxic hepatocarcinogens.

PL 877 REDOX STATES OF THIOREDOXIN-1 DIFFERENTIATE HUMAN CANCER FROM ITS ADJACENT BENIGN TISSUES.

W. Shan[1, 2], W. Zhong[2, 3] and T. D. Oberley[2, 3]. *[1]Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI, [2]Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI and [3]Pathology and Laboratory Medicine Service, William S. Middleton Veterans Memorial Hospital, Madison, WI.* Sponsor: C. Bradfield.

Our previous study using prostate cancer cell lines showed that thioredoxin 1 (Trx1) redox states could function as a biomarker of redox imbalance and was important in determining the response of prostate cancer cells to ROS-generating chemotherapeutic agents. In this study, we examined Trx1 redox states in several human cancer tissues including human kidney, liver and prostate cancer tissues. We found that Trx1 oxidation was consistently higher in cancer tissues tested compared to their adjacent benign tissues despite differential protein expression levels. The reduced form of Trx1 in benign tissues adjacent to tumors was lower in high-grade prostate tumors than low-grade prostate tumors. Our results suggested an oxidative environment in cancer tissues, and in high-grade prostate tumors, the adjacent benign tissues may be in precancerous oxidative stress conditions. The increased oxidation of Trx1 in cancer tissues was accompanied by a decrease in thioredoxin reductase 1 (TrxR1) protein levels, suggesting loss of TrxR1 may be a direct cause of increased Trx1 oxidation. This is the first study to detect Trx1 redox states in human tissues. Our results suggest that Trx1 redox states may be an important biomarker in determining the redox state of tissues and differentiates tumor tissues from their benign counterparts, and thus could be a useful adjunct for clinical diagnosis and treatment of cancer by analyzing and manipulating tumor redox states.

PL 878 TROVAFLOXACIN PROMOTES DNA DOUBLE-STRAND BREAKS IN A MACROPHAGE CELL LINE.

K. L. Poulsen[1, 2], K. Beggs[1, 2], R. Singhal[1], P. E. Ganey[1, 2] and R. A. Roth[1, 2]. *[1]Pharmacology and Toxicology, Michigan State University, East Lansing, MI and [2]Center for Integrative Toxicology, Michigan State University, East Lansing, MI.*

Trovafloxacin (TVX) is a drug with idiosyncratic drug-induced liver injury (IDILI) liability in human patients. A hepatotoxic interaction between bacterial lipopolysaccharide (LPS) and an otherwise nontoxic dose of TVX was established in a mouse model of IDILI. Characteristic of this model was a significant prolongation of the LPS-induced increase in tumor necrosis factor-alpha (TNF) concentration in the plasma of animals cotreated with TVX, and neutralization of TNF protected from TVX/LPS hepatotoxicity. In vitro, pretreatment of RAW 264.7 murine macrophage-like cells with TVX enhanced LPS-induced TNF release into cell culture supernatants. It has been previously suggested that TVX, a fluoroquinolone antibiotic that inhibits bacterial topoisomerase activity, inhibits eukaryotic topoisomerase. Since topoisomerase inhibitors and other agents that cause DNA damage can enhance inflammatory cytokine expression, we hypothesized that DNA strand breaks caused by inhibition of eukaryotic topoisomerase by TVX led to this enhancement of TNF production. In a cell-free system using recombinant human topoisomerase IIalpha, TVX (1 – 300 μM) increased retention of concatenated DNA and reduced the appearance of decatenated DNA, indicating the effectiveness of TVX as a topoisomerase II poison. The phosphorylation of histone H2AX (gammaH2AX) on Ser139 was evaluated by western blotting and used as a marker of DNA double-strand breaks (DSB). TVX (1 - 300 μM) caused a dose-dependent increase in gammaH2AX signal in RAW 264.7 cells within 2 hours. Increases in ubiquitinated H2AX were also observed, further implicating DSB as well as activation of DSB repair pathways. These findings suggest that TVX treatment results in DSB, which might activate macrophages to synthesize and release greater amounts of TNF in response to an inflammatory stimulus such as LPS. (Supported by NIH grant R01 DK061315 and T32 ES007255)

PL 879 EFFECT OF IONIZING RADIATION ON K- *ras* CODON 12 POINT MUTATIONS IN LUNG AND LIVER OF THE F344 RAT.

P. B. McKinzie, J. G. Shaddock, M. G. Pearce and V. N. Dobrovolsky. *Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Jefferson, AR.* Sponsor: B. Parsons.

Epidemiological studies indicate that ionizing radiation increases the incidence of human cancer. Previous studies have shown an increase in *hprt* point mutations with irradiation doses between 1-3 Gy. We examined whether 1-3 Gy whole-body irradiation of rats similarly induces K-*ras* codon 12 GGT to GAT mutations in lung and liver in an adjunct study of *Pig-A* mutation analysis. All animals were housed and treated according to the National Center for Toxicological Research Institutional Animal Care and Use Committee guidelines. Two-month old rats were exposed to 0, 1, 2, and 3 Gy delivered in 3 equal daily doses using an RS-2000 Biological Irradiator (Rad Source), and the organs harvested 16 weeks later. Genomic DNA from 0.5-1.5 g of tissue was isolated from each sample, and prepared for use in an Allele-Specific Competitive Blocker PCR (ACB-PCR) assay for K-*ras* codon 12 GGT to GAT mutation. The ACB-PCR assay can quantify base-pair mutations to a sensitivity of 1 mutant allele out of 100,000 wild-type alleles (10^{-5}) and was used to quantify the K-*ras* codon 12 GGT to GAT mutant fraction (MF) in the lung and liver of F344 rats exposed to X-irradiation. The geometric mean K-*ras* codon 12 GGT to GAT MF in lung tissue exposed to 0, 1, 2, and 3 Gy was 2.3×10^{-5}, 3.6×10^{-5}, 3.8×10^{-5}, and 4.1×10^{-5}, respectively, and in liver tissue the geometric mean MF was 7.9×10^{-5}, 14×10^{-5}, 4.6×10^{-5}, and 6.5×10^{-5} respectively. There was no statistical difference between any of the exposure groups, including the controls, for either tissue studied. The data suggests that X-irradiation does not induce K-*ras* codon 12 GGT to GAT mutation, a common cancer-associated oncogene mutation. We conclude that high levels of X-irradiation does not exert its carcinogenic effect through K-*ras* codon 12 GGT to GAT point mutations, which is consistent with ionizing radiation being classified as a clastogenic mutagen.

PL 880 ASSOCIATION OF ESTROGEN METABOLISM AND RISK OF NON-HODGKIN LYMPHOMA: DETECTION OF NOVEL BIOMARKERS FROM CASE-CONTROL STUDY.

L. Yang[1, 2], N. W. Gaikwad[3], D. D. Weisenburger[3], J. Vose[3], E. Rogan[3] and E. Cavalieri[3]. *[1]Department of Pharmacology and Chemical Biology, University of Pittsburtgh, Pittsburgh, PA, [2]Department of Environmental, Agricultural and Occupational Health, College of Public Health, University of Nebraska Medical Center, Omaha, NE and [3]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE.*

The precise molecular mechanism by which estrogens play a role in the initiation of non-Hodgkin lymphoma (NHL) has been in question. We hypothesize that specific estrogen metabolites, catechol estrogen-3,4-quinones [E1(E2)-3,4-Q], can bind with DNA to form 4-OHE1(E2)-N7Gua and 4-OHE1(E2)-N3Ade adducts; apurinic sites that are formed by depurination of these adducts can induce mutations by error-prone repair. When homeostasis of estrogen metabolism is disrupted and oxidative estrogen metabolism prevails in the body, then the risk of NHL will be increased. To test this hypothesis, NHL case-control study has been conducted to investigate how imbalance of estrogen metabolism affects the etiology of NHL and try to find potential early biomarkers in urine samples. This was accomplished

by partial purification of urine (from 15 male subjects with NHL and 30 male controls) with a solid phase extraction method and analysis by ultra-performance liquid chromatography/tandem mass spectrometry to assay 40 estrogen related compounds. The results show that there are significantly higher levels of depurinating estrogen-DNA adducts in the NHL cases than in the controls. In addition, the ratios of the sum of depurinating estrogen-DNA adducts to the sum of their corresponding estrogen metabolites and conjugates, which reflects the extent of imbalance of estrogen metabolism in the body, were significantly higher in the cases than in the controls. We conclude that formation of E1(E2)-3,4-Q, which arise from imbalance in estrogen metabolism, plays a very important role in the etiology of NHL. The ratios can be utilized as novel biomarkers to predict the risk and provide targeted strategies of NHL prevention in clinic settings.

R 881 CURRENT USES AND UNDERSTANDING OF THE TISSUE CROSS REACTIVITY ASSAY.

J. L. Bussiere[1], J. Cavagnaro[5], E. Galbreath[3], T. Machlachlan[6], N. Dybdal[4] and M. Leach[2]. *[1]Toxicology, Amgen, Inc., Thousand Oaks, CA, [2]Drug Safety Research and Development, Pfizer, Andover, MA, [3]Toxicology, Lilly Research Laboratories, Indianapolis, IN, [4]Safety Assessment, Genentech, Inc., South San Francisco, CA, [5]AccessBio, Boyce, VA and [6]Pharmacology and Toxicology, Genzyme, Framingham, MA.*

Tissue cross-reactivity (TCR) studies are screening assays conducted with monoclonal antibodies and related antibody-like biopharmaceuticals primarily to identify off-target binding, and secondarily to identify sites of on-target binding that were not previously identified. As presently utilized by the biopharmaceutical industry and regulatory agencies, TCR studies usually involve the ex vivo immunohistochemical (IHC) staining of a panel of frozen tissues from humans and animals. However, other methods of conducting TCR studies are possible. While ex vivo TCR studies have become routine in the development of antibody therapeutics, their value for the purpose of making safety assessment decisions by both industry and regulatory agencies has been questioned as experience with the assay has increased. Recently, an industry white paper was published to review the use of tissue cross-reactivity studies in the development of antibody-based biopharmaceuticals: history, experience, methodology, and future directions. In addition, a multinational pharmaceutical and biotechnology company survey was conducted to gain a better understanding of the use and value of the TCR assay in the development of biotherapeutic molecules. The information from this survey can be used to help understand the appropriate use and interpretation of this assay in a nonclinical drug development program. Our panel of experts will address the following issues of importance including how we got to where we are today; the technical aspects of the TCR assay and issues with interpretation; case studies and summary from white paper on use of TCR; industry survey results on use of the TCR assay; and, the new technologies for identifying off-target binding of monoclonal antibodies.

R 882 RISK AND RISK MANAGEMENT OF POTENTIALLY TOXIC COMPOUNDS FORMED BY COOKING FOOD.

S. J. Hermansky[1], W. Li[8], S. Saunders[2], N. Rachman[4], S. Olin[5], T. Troxell[3], M. Bolger[6] and A. Tritscher[7]. *[1]ConAgra Foods, Omaha, NE, [2]High Country Toxicology Group, Beech Mountain, NC, [3]Exponent, Washington, DC, [4]Grocery Manufacturer's Association, Washington, DC, [5]ILSI Research Foundation, Washington, DC, [6]U.S. FDA Center for Food Safety and Nutrition, Washington, DC, [7]World Health Organization, Geneva, Switzerland and [8]Frito Lay, Plano, TX.*

The discovery of acrylamide in food in 2002 by Swedish researchers initiated an international effort to assess exposure and manage risk to the public. Millions of dollars and countless hours of resources have been dedicated to this effort around the world. The concept that cooking changes the chemistry of food is not new but the presence of acrylamide across a wide variety of foods brought a new perspective to food safety for the international regulatory community. This issue continues to develop as different research groups focus on multiple heat-formed chemicals and various aspects of toxicology of these naturally occurring compounds. From the recent completion of the European Union HEATOX (Heat-Generated Food Toxicants: Identification, Characterization, and Risk Minimization) project to the development of increasingly sensitive and refined analytical methods that are able to detect ever lower concentrations of compounds in foods, it is becoming increasingly clear that acrylamide only represents one compound of many. This revelation presents challenges to both the scientific and regulatory communities. Just as importantly, resources are more frequently stretched thin as scientific debates are played out in the mass media and the credibility of the scientific and regulatory process is challenged. We will discuss the challenges and opportunities presented by risk management of compounds formed in food during cooking.

IS 883 EMERGING SCIENCE FOR ENVIRONMENTAL HEALTH DECISIONS: TOOLS, STRATEGIES, AND EVIDENCE.

W. H. Farland. *Colorado State University, Fort Collins, CO.*

The last year was very productive for the National Research Council's (NRC) Standing Committee on Emerging Science for Environmental Health Decisions, sponsored by the National Institutes of Environmental Health Science. A number of timely, topical sessions were held that included SOT members that were of special relevance to toxicology, risk assessment, and public health. These topics were designed to extend discussion contained in two 2007 NRC reports — Toxicity Testing in the 21st Century: A Vision and a Strategy and Application of Toxicogenomics Technologies to Predictive Toxicology and Risk Assessment. These sessions brought together government, industry, environmental groups, and the academic community to discuss emerging scientific concepts and advances and their potential implications for environmental health decisions. Specifically, these sessions have explored emerging tools and technologies for epigenetics, computational toxicology, stem cell models, and exposome research and their potential roles in identifying, quantifying, and mitigating environmental impacts on human health. In follow up to these dynamic sessions we will highlight many aspects of this important topic. Our panel of experts will address what was learned linking specifically to the common threads among the sessions, such as bioinformatics and expanding input information for systems approaches to toxicology. In closing, we'll synthesize how to best integrate data across these emerging and evolving areas of research and explore potential next steps for using such data and insights in environmental health decisions and policy.

S 884 METABOLIC BASIS OF RESPIRATORY TRACT CHEMICAL TOXICITY.

L. S. Van Winkle[1] and X. Ding[2]. *[1]Center for Health and the Environment/Veterinary Medicine: Anatomy, Physiology and Cell Physiology, University of California Davis, Davis, CA and [2]Division of Environmental Health Sciences, Laboratory of Molecular Toxicology, Wadsworth Center, New York State Department of Health, Albany, NY.*

The respiratory tract, including both the lung and the nasal tissue, has substantial metabolic activity, which can influence the distribution and action of drugs and xenobiotics, either inhaled or ingested. The metabolic enzymes that are active in the respiratory tract include cytochrome P450 monooxygenases, esterases, oxidoreductases, and dehydrogenases. Their distribution and activity can vary greatly with anatomic location, by species, sex and history of prior exposure. Respiratory tract metabolic activity can either enhance, or inhibit, local and systemic chemical toxicity. While this basic principle has been understood and investigated for many years, recent new approaches have allowed more in-depth investigation of the mechanisms of toxicity in the respiratory tract following exposure to bioactivated toxicants. Significant advances have been made, with the use of novel animal models, new detection methods, application of site-specific approaches to colocalize toxicity and metabolism, recombinant human enzymes, and modeling, which enhance our understanding of the contributions of xenobiotic metabolism in the respiratory tract to toxicity in humans. Several examples highlighting recent progress in this area will be presented.

S 885 ROLE OF CYP2A AND CYP2F ENZYMES IN RESPIRATORY TRACT CHEMICAL TOXICITY – INSIGHTS FROM P450 KNOCKOUT AND HUMANIZED MOUSE MODELS.

X. Ding[1, 2]. *[1]Laboratory of Molecular Toxicology, Wadsworth Center, New York State Department of Health, Albany, NY and [2]School of Public Health, State University of New York at Albany, Albany, NY.*

Microsomal P450 enzymes metabolize numerous xenobiotic compounds. Transgenic and knockout mouse models are valuable for determining P450s' roles in various environmental diseases. Cyp2a5 and Cyp2f2 are among a cluster of Cyp2a, 2b, 2f, 2g, 2s genes on mouse chromosome 7, many of which are expressed preferentially or abundantly in the respiratory tract. A Cyp2a5-null mouse and a Cyp2f2-null mouse have been produced and characterized recently in this laboratory. Initial application of these two mouse models to studies on xenobiotic metabolism has led to interesting findings on the roles of the respective P450 enzymes in the in vivo metabolism and respiratory tract toxicity of several important environmental chemicals. For examples, CYP2A5 was found to play a major role in the metabolic activation of NNK, a tobacco-specific nitrosamine, in both lung and nasal olfactory mucosa (OM); it also contributes to the metabolic activation of naphthalene and 3-methylindole in the OM, but not in the lung . On the other hand, CYP2F2 plays the major role in naphthalene metabolic activation in the

lung, as well as in the systemic clearance of naphthalene. The abilities of human CYP2A13 and CYP2F1 to metabolize various respiratory toxicants in vivo are also being determined, through the use of P450-humanized mouse models, generated by crossbreeding between a CYP2A13/2F1-transgenic mouse and the Cyp2a5-null or the Cyp2f2-null mouse. Additionally, other novel mouse strains, in which all or a part of the 1.4-Mb Cyp2a-2b-2f-2g-2s gene cluster is deleted, are being produced and characterized; these mouse strains would provide a clean (or cleaner) genetic background for humanized mouse models that express the orthologous human CYP2A, 2B, 2F1, or 2S1. Potential utility of the Cyp2a5-null, the Cyp2f2-null, and the Cyp2 cluster-null mouse models for in vivo studies on the metabolic basis of respiratory tract xenobiotic toxicity will be discussed.

S 886 METABOLISM OF INHALED GLUCOCORTICOIDS IN LUNG: SELECTIVE INACTIVATION OF CYP3A5.

G. S. Yost. *Deptartment of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.*

The major drug-metabolizing cytochrome P450 in the liver, CYP3A4, is not expressed in human lung, but CYP3A5 and CYP3A7 enzymes are highly expressed in human lung airway and parenchymal cells. One of the most frequently used inhalation glucocorticoids, fluticasone, is efficiently dehydrogenated to an electrophilic intermediate, which selectively inactivates CYP3A5, but not CYP3A7. Surprisingly, none of the other four major glucocorticoids inactivates the CYP3A4/5/7 enzymes, despite the highly similar structures of the glucocorticoids, and the fact that all five are dehydrogenated to similar electrophilic quinone methides by these enzymes. We have characterized the quinone methides, and their glutathione adducts, from incubations with purified CYP3A4/5/7 enzymes. In addition, we have found that the dehydrogenated intermediates were produced by epithelial cells that were obtained from tracheal washes of intubated preterm infants. Our current studies seek to determine if the electrophilic intermediates are cytotoxic to the primary lung epithelial cells from these children. The partition ratio of 3A5 inactivation by fluticasone is 3, so fluticasone is one of the most efficient inactivators of any P450 enzyme. It is possible that repeated use of inhaled fluticasone by asthmatics could inhibit local metabolism and increase systemic concentrations. Disposition of some inhaled glucocorticoids is postulated to be solely dependent on esterase activities, but we have shown that a moderate but important percentage of the esterase activities was catalyzed by the CYP3A enzymes. Thus, induction or inhibition of these P450s could significantly change effective concentrations of specific glucocorticoids in targeted respiratory cells. Supported by NIH Grants GM074249 and HD060559.

S 887 SEX DIFFERENCES IN METABOLISM AND TOXICITY IN THE LUNG.

L. S. Van Winkle, K. Sutherland, D. Morin, P. Edwards and A. Buckpitt. *University of California Davis, Davis, CA.*

Sex differences in metabolism of xenobiotics have been well established for compounds metabolized by the liver, yet there have been few studies of sex differences in pulmonary metabolism and toxicity. Naphthalene (NA) is bioactivated by cytochrome P450 monooxygenases and causes airway epithelial cytotoxicity. Using airway microdissection and laser scanning confocal microscopy, specific sites of focal epithelial damage can be mapped within the respiratory tract. Metabolism in target vs non target sites can be measured. Both estrous cycle as well as repeated exposure to NA alters the pattern of toxicity in airways of female mice. Differences in activation of NA between sexes are not sufficient to account solely for susceptibility. This raises the possibility that key detoxification mechanisms such as conjugation to glutathione or further metabolism of the epoxide by epoxide hydrolase, formation of non-traditional metabolites or differences in levels of delivered naphthalene are key to the toxicity and might differ by sex. We have explored these concepts using a combination of site specific approaches, new methods to measure naphthalene and mice deficient in microsomal epoxide hydrolase (mEH KO). mEH KO female mice have a different pattern of cytotoxicity to inhaled NA than wild type (WT) female mice. Toxicity was not markedly different in WT and KO mice but varied by anatomic location. In mEH KO mice the lobar bronchus was slightly resistant to injury and the terminal bronchiole was more slightly more susceptible than in WT female mice. These results indicate that NA metabolites downstream of the NA dihydrodiol likely contribute little to overall epithelial cytotoxicity. In a separate study, female mice exposed repeatedly to NA have a different pattern of response to a high challenge dose: terminal bronchioles developing a tolerant phenotype but more proximal airways remaining susceptible. These studies support that metabolism and detoxification mechanisms vary by site. Understanding of this variation is key to defining differences in susceptibility between males and females. Support: ES04311, ES04699, ES012720

S 888 DEVELOPMENT OF ADDUCTED PEPTIDE BIOMARKERS TO ADDRESS THE CONSEQUENCES OF HUMAN EXPOSURE TO RODENT PULMONARY TOXICANTS.

A. Buckpitt, N. Pham, D. Krawiec, D. Morin, W. Jewell and L. Van Winkle. *University of California Davis, Davis, CA.*

Despite 40 years of research into the role of reactive metabolites in cell injury and the identification of numerous protein adducts derived from a number of hepatic and pulmonary toxicants, comprehensive understanding of the role of specific adducts in cell injury remains elusive. Identification of key adducts and precursor reactive metabolites associated with cellular injury processes would improve the extrapolation of data obtained in animals to humans and will assist in identification of susceptible populations. Accordingly, we have focused on cross species comparisons of adducts and their precursor metabolites generated from the metabolically activated, environmental lung toxicant, naphthalene (NA). These studies showed that 1) adduction occurs with both high and low abundance proteins resulting in a wide range of specific activities of adducts, 2) many of the adducted proteins are identical to those previously identified in comprehensive studies on heptotoxic agents, and 3) rates of metabolism of NA to generate adducts are very similar in target tissues of rodents and non human primates. To address the issue of which protein adducts are critical, we are comparing adducted proteins from the non toxic glutathione depletor, diethyl maleate (DEM) to NA. Levels of adduct formation and persistence of adducts observed in pulmonary epithelium in vivo are nearly identical following doses which produce equivalent levels of glutathione depletion. Isotopic signatures (D0/D8) have been used to identify a number of peptide adducts in the urine of NA-treated animals. Neutral loss fragments indicate that the majority of these arise from NA oxide. There was no indication of quinone-derived adducted peptides in the urine. These studies support the view that there are critical adducts which lead to irreversible cell injury and that monitoring adducted peptides in the urine may be a novel, noninvasive approach for evaluating the formation of reactive metabolites in vivo which could be used in animal models and in humans. Supported by NIEHS 04311 and 04699.

S 889 RESPIRATORY TRACT METABOLISM AND INHALATION DOSIMETRY OF VAPORS.

J. B. Morris. *Toxicology Program, University of Connecticut, Storrs, CT.*

Inhalation dosimetry of vapors, particularly in the rodent, can be strongly influenced by local metabolism within respiratory tract tissues. By acting to lower the concentration of parent vapor molecules in respiratory tract tissues local metabolism serves to enhance vapor uptake efficiencies in the airways. To date the most extensive studies have been performed on upper airways. Metabolism via many enzymes has been shown to enhance vapor uptake in these airways including, CYP450, alcohol dehydrogenase, aldehyde dehydrogenase, and carboxylesterase. For high capacity enzymes such as carboxylesterase as much as one-third of the inspired burden is metabolized in a first pass fashion within nasal tissues of rodent. In vivo metabolism kinetics can be inferred from inspired vapor uptake studies by including animals treated with metabolic inhibitors (e.g. metyrapone or 5-phenyl-1-pentyne for CYP450). The Km values so obtained agree well with those obtained in vitro. Saturation of vapor metabolism occurs at high exposure concentrations leading to non-linear vapor uptake kinetics. This has been shown for numerous vapors including acetaldehyde (aldehyde dehydrogenase substrate vapor) and styrene and naphthalene (CYP450 substrate vapors). The exposure concentrations used in inhalation toxicity bioassays are typically sufficiently high to result in non-linear uptake due to enzyme saturation making extrapolation of such results to lower, more environmentally relevant concentrations problematic. Moreover marked species differences often exist in metabolic potential of the rodent versus man. Physiologically based pharmacokinetic models for rodent and human upper airway uptake and metabolism of inspired vapors have been developed and validated. Such models provide a comprehensive way to perform high-to-low dose as well as species extrapolations. Recently these modeling approaches have been extended to include the entire respiratory tract. Such models provide a framework to interpret the role of metabolism in influencing vapor uptake and dosimetry throughout the entire respiratory tract of the rodent as well as the human.

S 890 STEM CELL AND CELL THERAPY APPROACHES FOR UNDERSTANDING CELLULAR INJURY AND WOUND HEALING IN DERMAL, OCULAR, AND LUNG INJURY.

J. J. Yourick. *Medical Chemical Countermeasure, Defense Threat Reduction Agency, Fort Belvior, VA.*

Thermal and chemical burns as well as chemical injury to the skin, eye and lung invoke a vast tissue and cellular response followed by the initiation of wound healing mechanisms. Stem cells may be defined as undifferentiated cells that have the ca-

pacity for self-renewal and may differentiate into many different cell types when stimulated by specific cellular signals. Injury to stem cell tissue populations has immense implications for normal repair and restoration of tissue function after chemical injury. Research on stem cells, such as epidermal stem cells, dermal stem cells, mesenchymal stem cells and embryonic stem cells all have potential to repair and restore structure and function to the skin, lung and eye after extensive injury. Using a rabbit ocular model, the pathology of limbal stem cell deficiency has been described for the cytotoxic effects of sulfur mustard on epithelial stem cells. The damage creates a secondary process at the niche leading to slow stem cell death. Potential ocular treatment modalities and the implications of treatment will be discussed for these surface epithelia cells. The application of keratinocytes in suspension has been shown to improve epidermal wound healing in pig and mouse models. Following processing of a skin biopsy after a dermal injury, the resulting cell suspension (ReCell® Autologous Cell Harvesting Device) provides autologous keratinocytes, epidermal stem cells, melanocytes, fibroblasts and Langerhans cells for application onto prepared wound beds to facilitate healing. Effort has gone into identifying stem cells and characterizing their function in tracheobronchial, bronchiolar and alveolar compartments of the lung. The use of these isolated stem cell populations has shown promise in mitigating lung injury. Mesenchymal stem cells have been isolated from leukopak by magnetic separation and are being cultured for the treatment of sulfur mustard skin injury. In addition, the pharmacological modulation of stem cells or their function to treat skin injuries may provide new treatment strategies. An overview of recent advances in dermal, ocular and pulmonary induced injury related to stem cells will be presented along with potential stem cell and other cell-based therapies as relates to tissue repair and wound healing.

S 891 THE INVOLVEMENT OF EPITHELIAL STEM CELLS IN THE PATHOGENESIS OF SULFUR MUSTARD INJURIES: MECHANISM AND POTENTIAL THERAPY.

T. Kadar, V. Horwitz, L. Cohen, M. Cohen, R. Sahar, H. Gutman, L. Tveria, R. Gez, S. Dachir and A. Amir. *Pharmacology, Israel Institute for Biological Research, Ness Ziona, Israel.* Sponsor: J. Yourick.

Sulfur mustard(SM) is a potent vesicant, known to cause prolonged injuries to the eyes, skin and respiratory system. Ocular injuries following SM exposure are characterized by an acute phase expressed by inflammation of the anterior segment and corneal erosions, and by delayed corneal injuries characterized by epithelial defects, chronic inflammation and vascularization. Using our rabbit ocular model, we defined the late pathology as Partial Limbal Stem Cell Deficiency (PLSCD). Yet, the involvement of epithelial stem cells (SC) in the development of SM injuries in the cornea, as well as in other epithelial tissues is still elusive.
The present study aimed to investigate the pathological mechanism underlying SC deficiency associated with SM injury and to test potential therapy. Epithelial SC were identified by immunohistochemistry and RT-PCR of ABCG2 and p63, and by in vivo BrdU labeling of slow cycling cells.
The results indicated that corneal epithelial SC, residing in the limbus, were not affected primarily by SM, however, prolonged damage of nerves and inflammatory response were shown in the limbal niche, in eyes displaying the late pathology. Thus, the deficiency of epithelial SC was not derived from a cytotoxic effect of SM on the SC, but due to pathological processes in the SC niche that contributed later-on to a slow death of the SC and to development of LSCD.
These findings point toward the use of anti-inflammatory drugs and trophic factors for prevention of the damage in the niche and consequently the loss of SC. Cellular therapy by transplantation of limbal explants is suggested as a symptomatic treatment for eyes displaying LSCD. These results and in particular the absence of a primary cytotoxic effect of SM on epithelial SC indicate the presence of a therapeutic window for intervention and have implication on other surface epithelia, vulnerable to SM toxicity.
Part of this work was supported by the U.S. Army Medical Research and Material Command under award # W81XWH-08-2-0129.

S 892 CELL-BASED THERAPEUTICS IN BURN MEDICINE.

J. S. Graham. *U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.* Sponsor: J. Yourick.

New strategies using skin substitutes and autologous or allogeneic cell suspensions have been developed to improve healing of burn injuries. Skin substitutes grown in a laboratory and populations of individual skin cells harvested from biopsies can be applied to debrided burns to return damaged skin to optimal cosmetic appearance and normal function in the shortest period of time. The use of skin substitutes to temporarily restore the multiple functions of normal skin has been of substantial benefit in the management of cutaneous burn injuries. Skin substitutes are widely used in the management of human thermal burns and can be (1) temporary or permanent, (2) epidermal, dermal, or composite, and (3) biologic or synthetic. They have also been shown to be effective in speeding up time to closure of chronic leg and foot ulcers, surgical excision sites, and partial-thickness donor sites. The application of keratinocytes in suspension has been shown to improve epidermal wound healing in pig and mouse models. ReCell® Autologous Cell Harvesting Device (Avita Medical Americas LLC, Woburn, MA) is an innovative medical device that was developed to allow rapid harvesting of autologous cells from a thin split-thickness biopsy. Harvesting is followed by spray application of a population of skin cells onto wounds within 30 min of collecting the biopsy, without the need of culturing the cells in a clinical laboratory. Following processing of the skin biopsy, the resulting cell suspension provides autologous keratinocytes, epidermal stem cells, melanocytes, fibroblasts, and Langerhans cells for application onto prepared wound beds. An overview of cell-based therapeutics in treating cutaneous vesicant injuries will be provided, to include a review of available products and use of several of these products in treating sulfur mustard injuries in a weanling swine model.[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 the Care and Use of Laboratory Animals (National Research Council, 1996), and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.]

S 893 STEM CELLS AND DAUGHTER CELLS IN REGENERATION OF EPITHELIAL SURFACES.

M. Simon[1], J. Tafrova[1], L. Wan[1], J. S. Graham[2], J. Azeke[2], E. Braue[2] and S. McClain[3]. [1]*Stony Brook University, Stony Brook, NY,* [2]*U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD and* [3]*McClain Laboratories, LLC, Smithtown, NY.* Sponsor: J. Yourick.

Subsequent to technologies allowing in vitro expansion of keratinocytes, groups in multiple countries have used cultured epithelia in suspension or as tissue to treat partial and full-thickness cutaneous and ocular surface injuries. Although mesenchymal-derived factors can determine lineage, when engrafted cultured epithelia re-express body site specific markers lending support to the notion that cells in culture maintain the ability to express stemness in vivo. Unresolved is the question of the identity between the in vivo and in vitro stem cell populations. However, multiple studies support the notion that microenvironment regulates the stem cell compartment. Transit amplifying cells may exit the cell cycle and either re-enter the stem cell compartment or differentiate, and differentiated cells may dedifferentiate to regenerate viable tissue by first expanding the stem cell compartment. Irrespective of the mechanisms regulating the size and origin of the epithelial stem cell pool, factors derived from cultured epithelia enhance both keratinocyte and fibroblast proliferation and migration, and thereby provide the required mesenchymal support and functionally relevant epithelial stem cells for wound resurfacing. In vitro studies show that these factors show benefit in a background of media replete with epidermal growth factor, cholera toxin, insulin, and hydrocortisone. Under specific condition, when lyophilized the growth promoting and chemotactic activities are stable for >one month (limit of experimental testing) at temperatures up to +50oC and when rehydrated are stable at 37oC for up to 2-days. In light of the stability profile and capacity to support expansion and migration of required epithelial and mesenchymal cells, this material may offer therapeutic benefit to individuals with partial thickness chemical or thermal burns, who may be housed in environmentally controlled conditions or are in the extreme conditions can be experienced in the field.

S 894 EMBRYONIC, MESENCHYMAL, AND PHARMACOLOGICAL MODULATION OF STEM CELLS FOR THE TREATMENT OF CUTANEOUS VESICANT INJURY.

M. P. Nambiar. *Closed Head Injury, Walter Reed Army Institute of Research, Silver Spring, MD.*

Exposure to sulfur mustard (HD) leads to significant loss of skin stem cell keratinocytes that are required for constant supply of epidermal layer, prevent scar formation/skin contraction, hair growth and cosmetic recovery. Superficial skin injuries following HD exposure have higher significance on the battlefield and may not likely require surgical approaches. We have developed bioengineered skin from mouse embryonic stem cells by air/liquid culture and found that it protects against half-mustard induced cutaneous vesicant injury using a mouse model. Cryopreserved bioengineered skin also protect against half-mustard skin injury.

Although embryonic stem cells are ideal for allograft treatment, there are limitations. Allogenic mesenchymal stem cells (MSCs) from human peripheral blood/bone marrow or cord blood are less prone to trans-differentiation and may provide more practical approach for the treatment of HD exposure. MSCs have the ability to grow in culture, generate hair follicles and sweat glands. They can be infused or applied directly to the site of injury. MSCs are being isolated by magnetic separation, cultured and characterized for the treatment of HD skin injury. Another potential new strategy for the treatment of vesicant injury is pharmacological modulation of stem cells or their function. Pharmaceutical agents can be administered immediately after HD exposure to significantly reduce the injury/promote healing. Colony stimulating factors, statins, angiotensin-II receptor antagonists/angiotensin convertible enzyme inhibitors, erythropoietin, nitric oxide donors, estrogen and glitazones enhance stem cell survival and mobilize stem cells from bone marrow. Chemokine receptor antagonists, glycogen synthase kinase inhibitors and histone deacetylase inhibitors modulate the growth, differentiation and mobilization of stem cells. We are evaluating the efficacy of pharmacologic agents that enhance stem cell survival, self renewal and mobilization for cutaneous HD injury.

S 895 CONTROL OF STEM CELL DIFFERENTIATION IN THE LUNG.

J. D. Laskin[1, 3] and D. L. Laskin[2, 3]. [1]*Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ,* [2]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and* [3]*Environmental and Occupational Health Sciences Institute, Piscataway, NJ.*

It is well recognized that mesenchymal stem cells are immunomodulatory. Although the precise mechanisms of immune regulation are not known, these cells have been shown to secrete a variety of antiinflammatory growth factors, lipid mediators, and cytokines and have the capacity to suppress the immune system and stimulate reparative processes. The immunosuppressive functions of mesenchymal stem cells can also be stimulated by proinflammatory mediators including interferon-γ in combination with other cytokines such as tumor necrosis factor-α, interleukin-1α, or interleukin-1β. These cytokines drive T cell migration into the proximity of mesenchymal stem cells which suppress their functioning. They also regulate macrophage functioning, stimulating the conversion of M1 classically activated macrophages, which display a cytotoxic, proinflammatory phenotype, into alternatively activated M2 macrophages, which suppress immune and inflammatory responses and participate in wound repair and angiogenesis. Mesenchymal stem cells also have the capacity to differentiate into a variety of lung cell types which contribute to the repair of damaged tissues. It is these properties that make mesenchymal stem cells attractive for use in clinical situations where it would be of interest to stimulate repair of diseased or injured tissues. Much effort has gone into identifying stem cells and characterizing their functioning in tracheobronchial, bronchiolar and alveolar compartments of the lung. This has been aided by the development of experimental systems where exposure to chemical toxicants including ozone and mineral fibers can stimulate stem cell proliferation and polarization. The use of these isolated stem cell populations has shown promise in mitigating chemical-induced lung injury by stimulating normal lung cell proliferation and differentiation. This work was supported by NIH Grants HL096426, AR055073, GM034310, ES004738, CA132624, and ES005022.

S 896 UNCOVERING THE ROLE OF NON-CODING RNAS IN TOXICOLOGY.

M. E. Hahn[1] and T. Tal[2]. [1]*Biology, Woods Hole Oceanographic Institution, Woods Hole, MA and* [2]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

Over the past ten years non-coding RNAs (ncRNAs) have emerged as pivotal players in fundamental physiological and cellular processes and have been increasingly implicated in cancer, immune disorders, and cardiovascular, neurodegenerative, and metabolic diseases. MicroRNAs (miRNAs) represent a class of ncRNA molecules that are predicted to post-transcriptionally regulate the expression of 30-60% of all human protein-coding genes and as such, miRNAs play key roles in cellular and developmental processes, human health, and disease. Recently, miRNAs have surfaced as targets of developmental, hepatic, neurological, and carcinogenic toxicological agents, and have increasingly been identified as putative regulators of phase I xenobiotic-metabolizing enzymes. We will highlight impactful research demonstrating the growing understanding of the role of miRNAs in toxicological modes of action, study the mechanisms of miRNA-mediated toxicity in a variety of emerging model systems.

S 897 EXPLORING THE ROLE OF MICRORNAS AS MEDIATORS OF DEVELOPMENTAL NEUROBEHAVIORAL TOXICITY IN ZEBRAFISH.

T. L. Tal[1], J. A. Franzosa[1], S. Tilton[2], K. Waters[2] and R. L. Tanguay[1]. [1]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR and* [2]*2Computational Biology & Bioinformatics Group, Pacific Northwest National Laboratories, Richland, WA.*

MicroRNAs (miRNAs) are non-coding RNAs that direct post-transcriptional repression of protein coding genes. While miRNA biogenesis is critical to nervous system development, the neurobehavioral function of miRNAs is unknown. Prenatal alcohol exposure results in fetal alcohol syndrome disorders (FASD). Epidemiological studies suggest that the majority of children with FASD present with deficits in neurobehavioral function in the absence of clinically discernable physical abnormalities. Previous automated zebrafish neurobehavioral assessments showed that transient developmental exposure to ethanol results in reductions in locomotor activity relative to unexposed controls. To identify miRNAs and their putative mRNA targets that are misexpressed following developmental ethanol exposure, miRNA and mRNA microarray analyses were conducted. At developmental stages consistent with ethanol-mediated neurobehavioral abnormalities, expression of multiple CNS-enriched miRNAs were significantly reduced. mRNA array data was queried to identify transcripts that were elevated upon exposure to ethanol that also contained miRNA-specific recognition elements in their 3'UTRs. Subsequent computational analyses revealed that ethanol exposure disrupts expression of miRNAs that direct neurogenesis. To examine the functional role of ethanol-sensitive miRNAs, antisense oligonucleotide morpholinos were used to transiently knockdown miRNA expression during development. Knockdown of multiple CNS-enriched miRNAs phenocopied ethanol-induced behavioral hypoactivity. These data indicate that ethanol exposure misregulates the expression of miRNAs that collectively choreograph nervous system development and function and support the concept that miRNA signaling pathways are targets of developmental neurotoxicants. This research was supported by P30ES00210, NIEHS T32ES07060, and the OSU Linus Pauling Institute.

S 898 DEFINING THE DEVELOPMENTAL ROLE OF MICRORNAS IN ORCHESTRATING TOXICOLOGICAL RESPONSES.

J. A. Franzosa, T. L. Tal and R. L. Tanguay. *Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.*

microRNAs (miRNAs) are a class of small non-coding RNAs that exert post-transcriptional control over gene expression. To obtain a complete picture of the molecular responses that lead to biological perturbations, it is critical to consider the potential role of miRNAs in toxicity mechanisms. The genetic tractability of zebrafish renders them an exceptional model for evaluating the function of miRNAs in mediating teratogenicity. In this study, retinoic acid (RA) was used to investigate whether miRNAs are targets of toxicants. Improper spatial and temporal regulation of RA signaling during development results in teratogenic outcomes. Previous data demonstrated that developmental RA exposure results in body axis defects, suggesting that RA exposure negatively impacts somitogenesis. To determine whether specific miRNAs are misexpressed following RA exposure, microarray analyses were conducted. Strikingly, the expression of three miR-19 family members was significantly repressed by RA exposure during somitogenesis. Bioinformatics analyses predicts that miR-19 targets cyp26a1, a key enzyme responsible for converting RA into its inactive form. A physiological reporter assay was used to confirm that cyp26a1 is a bona fide target of miR-19 in vivo. Antisense oligonucleotide morpholino repression of miR-19 expression phenocopied the effects of RA exposure. Furthermore, cyp26a1 expression during somitogenesis was increased in RA exposed embryos and miR-19 morphants. In gain-of-function studies, microinjection of miR-19 mimic rescued the axis defects induced by RA exposure. Together, these results indicate that the teratogenic effects of RA exposure result, in part, from repression of miR-19 expression and the subsequent misregulation of cyp26a1. This research demonstrates the strength of zebrafish as a model for investigating the functional role of miRNAs in the misregulation of molecular pathways by toxicants during development. This research was supported by NIH P30ES00210, NIEHS T32ES07060, and OSU Linus Pauling Institute Pilot Project Grant.

S 899 IDENTIFICATION OF NEW NON-CODING SMALL RNA IN BREAST CANCER.

C. Rovira. *Clinical Sciences, Department of Oncology, Lund University, Lund, Sweden.* Sponsor: T. Tal.

To comprehensively characterize the set of miRNAs involved in breast cancer we used next-generation sequencing and generated millions of small RNA reads from cell lines and tumors with paired samples of normal and tumor-adjacent breast tis-

sue. The depth of our analysis led to the discovery of an unexpectedly large number of hundreds of new, well-supported miRNA precursors, corresponding to almost 50% of the total number of human miRNAs deposited in the miRNA registry. Most of the mature miRNAs encoded by these new precursors had low expression in our samples, but can be detected in other human tissues. 49% of these new genes were shown to be associated with Argonaute proteins, the main components of the RNA silencing machinery, confirming that they are new functional genes. Interestingly, more than one tenth of the new miRNAs are located in regions that are genomically unstable in breast cancer.
The sequence data set also include non-canonical miRNAs produced by alternative mechanisms. We found that the vault RNA (vRNA) may act as a pre-miRNA. Vault particles are conserved organelles that have been implicated in multidrug resistance and intracellular transport. They are formed by three different proteins and the non-coding vault RNAs. We showed that human vRNAs are digested by Dicer to produce several small RNAs (svRNAs). At least one of these svRNAs, we named svRNAb, associates with Argonaute proteins to guide sequence-specific cleavage and regulate gene expression in a manner similar to miRNAs. We also demonstrate that svRNAb downregulates CYP3A4, a key enzyme in drug metabolism. Our findings expand the repertoire of small regulatory RNAs and assign, for the first time, a function to vRNAs that may help explain the observed association between vaults and drug resistance.

S 900 ONCOGENIC MICRORNAS AS DRUG TARGETS FOR CANCER CHEMOTHERAPY.

S. Safe. *Veterinary Physiology, Texas A&M University, College Station, TX.*

MicroRNAs (miRs) are small non-coding RNAs (18-24 nucleotides) which primarily function to inhibit mRNA expression and it has been estimated that at least 30% of protein-encoding genes are regulated by miRs. In cancer, some miRs can exhibit tumor suppressor- or oncogenic-like activity and therefore become potential targets for cancer chemotherapy. Specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 are overexpressed in cancer cells and tumors, and Sp-regulated angiogenic (VEGF and its receptors), growth promoting (cyclin D1, c-Met and EGFR) and survival (bcl-2 and survivin) genes are important pro-oncogenic factors. High expression of Sp transcription factors and Sp-regulated genes has been linked to miR-mediated inhibition of Sp repressor genes. MiR-27a is part of a miR cluster and inhibits expression of ZBTB10, an Sp repressor, and transfection of cancer cells with antisense-miR-27a or ZBTB10 expression plasmid decreases expression of Sp1, Sp3, Sp4 and Sp-regulated genes. Drugs that modulate the miR-27a:ZBTB10-Sp axis have now been identified and these agents simultaneously decrease multiple Sp-regulated genes that are themselves individual targets for cancer chemotherapy. Sp transcription factors and Sp-regulated genes also are repressed by other miRs including the miR-17-92 cluster, and results of ongoing studies suggest that overexpression of Sp genes in tumors may be due to multiple miR-Sp repressor interactions which are also targets for anticancer drugs. Recent studies have identified other non-coding RNAs (ncRNAs) that modulate chromatin structure and carcinogenesis, and several agents that modify miRs also affect expression of specific long ncRNAs. Preliminary results on drug-ncRNA interactions will be discussed.

S 901 MICRORNA REGULATION OF DEP-INDUCED INFLAMMATION IN AIRWAY EPITHELIAL CELLS.

M. J. Jardim, L. Dailey and D. Diaz-Sanchez. *U.S. EPA, Chapel Hill, NC.*
Sponsor: T. Tal.

Morbidity and mortality attributable to air pollution continues to be a growing problem worldwide. Despite several studies on the health effects of ambient air pollution, underlying molecular mechanisms of susceptibility and disease remain elusive. The epigenome controls gene expression without affecting the DNA sequence itself and it can be inherited from generation to generation. It is not currently understood how exposure to air pollutants can modify underlying epigenetic mechanisms to elicit cellular responses such as inflammation. MicroRNAs are small non-coding RNAs that have been quickly established as key regulators of gene expression. As such, miRNAs have been found to control several cellular processes including apoptosis, proliferation and differentiation. We have previously shown that exposure to diesel exhaust particles (DEP) induces a rapid change in the expression of several microRNAs in human bronchial epithelial cells (BECs). Molecular network analysis of hsa-miR-513 suggested that the putative targets of this miRNA were enriched for regulators of the inflammatory response, including IL8 and COX2. We hypothesized that this miRNA plays an important role in attenuating the inflammatory response after exposure to DEP by regulating IL8 and COX2 mRNA levels. Indeed, over-expression of miR-513a leads to an attenuation of DEP induced IL8 and COX2. Alternatively, induced knockdown of this microRNA resulted in heightened levels of IL8 and COX2 mRNA in the presence of DEP, suggesting that miR-513a may be acting as a negative regulator of the inflammatory response. Furthermore, induction of miR-513a upon stimulation with DEP implies that it may play a role in a negative feedback loop which serves to attenuate the inflammatory response once critical levels of inflammatory mediators have been achieved. This abstract of a proposed presentation does not necessarily reflect EPA policy.

W 902 IDENTIFICATION OF CHEMICAL RESPIRATORY ALLERGENS: PRINCIPLES AND NEW DEVELOPMENTS.

I. Kimber[1] and T. Yoshida[2]. [1]*Faculty of Life Sceinces, University of Manchester, Manchester, UK, United Kingdom and* [2]*Asahikawa Medical College, Asahikawa, Japan.*

There are several new developments and opportunities in chemical respiratory allergy. This is an important occupational health problem associated with significant morbidity, and occasionally mortality. The identification and characterization of chemical respiratory allergens has presented toxicologists with some significant challenges, not least because there remains uncertainty about the immunological mechanisms that may result in allergic sensitization of the respiratory tract. Moreover, there is continuing debate about the relevant routes of exposure for the acquisition of sensitization. Previously attention focused primarily on the development of predictive test methods based upon animal, mainly guinea pig and mouse models, or through exploitation of (quantitative) structure-activity relationships. More recently, however, other strategies have been proposed and developed, included among which are modified peptide reactivity assays, and the identification of altered gene expression signatures specific for chemical respiratory allergens. Recent progress will be reviewed critically and prospects for the development of widely accepted methods for the identification and characterization of chemical respiratory allergens will be discussed.

W 903 IDENTIFICATION AND CHARACTERIZATION OF CHEMICAL RESPIRATORY ALLERGENS:CHALLENGES AND OPPORTUNITIES.

I. Kimber. *Faculty of Life Sceinces, University of Manchester, Manchester, UK, United Kingdom.*

It is now well established that a number of chemicals have the potential to induce allergic sensitization of the respiratory tract, associated with occupational asthma. Chemical respiratory allergy poses a number of important and intriguing challenges to toxicologists, and there are a variety of hurdles that must be negotiated if robust and reliable methods for hazard identification and characterization are to be developed. In this opening presentation an historical perspective of approaches used for the toxicological evaluation of respiratory sensitising chemicals will be provided, with a critical appraisal of the strengths and limitations of methods that have been proposed. In addition, the immunological mechanisms that result in the development of allergic sensitization of the respiratory tract will be reviewed, and areas of uncertainty highlighted and discussed. Consideration will be given also to the relevance of IgE antibody responses for chemical respiratory allergy, and the routes of exposure that will support the acqisition of sensitisation. Concluding comments will focus on the opportunities that now exist for significant progress to be made.

W 904 ANIMAL MODELS OF CHEMICAL RESPIRATORY ALLERGY.

J. Pauluhn. *Toxicology, Bayer HealthCare, Wuppertal, Germany.*

Occupational exposure priming and eliciting respiratory allergy can be attributed to two routes: the skin and the respiratory tract. Especially in context with uncontrolled accidental exposures, doses high enough to prime sensitization may more readily be delivered to the skin than to the respiratory tract. Skin exposures may lead to priming responses rendering the respiratory system more susceptible to subsequent inhalation exposures and ensuing allergic response (asthma). This complex inter¬relationship was investigated in a Brown Norway (BN) rat skin and inhalation induction asthma model followed by a dose-escalation-like inhalation challenge protocol. The latter serves the purpose to estimate the non-elicitation threshold concentration in sensitized, asthmatic rats. Independent on the route of induction, this was followed by three subsequent fixed concentration inhalation challenges to the sensitizing chemical in intervals of 2 weeks. At the fourth challenge, a dose-escalation regimen was used to determine the elicitation threshold on 'asthmatic' rats. Response was characterized by inflammatory endpoints in bronchoalveolar lavage (BAL), supplemented by physiological measurements with focus

on respiratory changes immediate and delayed in onset. Independent on the mode of induction, response was characterized by increased influx of neutrophilic granulocytes (PMN) and delayed respiratory response. BN rats induced by the high C x short t protocol elaborated the most pronounced influx of PMNs and delayed-onset responses. No route-of-induction related differences in elicitation thresholds were found. This data suggest that the vigor of asthma-like responses appears to support the well established dose-of-induction paradigm. The more pronounced elicitation response following topical induction is consistent with the appreciably higher dose that can be delivered to the skin as compared to the respiratory tract. Likewise, inhalation induction becomes more efficient using a high C, short t exposure regimen which is consistent with the common view held that intermittent peak exposures are important contributing factors in the ontogenesis of this occupational disease.

W 905 PEPTIDE REACTIVITY OF CHEMICAL RESPIRATORY ALLERGENS.

J. F. Lalko[1, 2]. [1]*The University of Manchester, Manchester, United Kingdom and* [2]*Research Institute for Fragrance Materials Inc., Woodcliff Lake, NJ.* Sponsor: I. Kimber.

A common characteristic of all chemical allergens, both respiratory and skin, is the ability to form stable associations with proteins. The covalent modification of nucleophilic amino acids by electrophilic chemical allergens is a key step in the sensitization process; with the resulting non-self, hapten-protein complex being sufficient to provoke an immune response. In recent years this mechanistic understanding has been exploited to develop in vitro screening assays to identify potential skin sensitizers. Typically, these Peptide Reactivity Assays (PRAs) are conducted by incubating the test chemical in the presence of a model peptide and characterizing the reaction by measuring the depletion of the nucleophile and/or the formation of adducts (via HPLC UV and/or MS detectors of various configurations). These developments have focused primarily on the identification of skin sensitizers; however, there is interest in applying similar approaches to the identification of chemical respiratory allergens. The available information, though limited, shows that both chemical respiratory and skin allergens result in positive responses for reactivity in PRAs. From a risk assessment and management perspective, it is advantageous to separate chemicals based on their potential to act as either skin or respiratory allergens. Some investigators have pointed to selective modification of specific amino acids or proteins (i.e. comparing cysteine to lysine; or cell vs. serum associated protein binding) as contributing to the ability of an allergen to result in one or the other form of sensitization. Overall, experience to date indicates that chemical reactivity measurements can provide useful information and have the potential to form a key component of future integrated testing strategies to characterize potential respiratory allergens. Such opportunities will be surveyed against a background of the immunobiology of allergic sensitization and current state-of-the-art approaches to the measurement of peptide/protein reactivity.

W 906 GENE EXPRESSION CHANGES AND THE IDENTIFICATION OF CHEMICAL RESPIRATORY ALLERGENS.

D. R. Boverhof. *The Dow Chemical Company, Midland, MI.*

There is a need for experimental approaches for the identification and characterization of chemicals with the potential to induce respiratory allergy. Currently there is uncertainty with regards to the mechanisms involved in the development of respiratory allergy and their distinctions from contact allergy. Toxicogenomic technologies allow for global profiling of gene expression in response to xenobiotic exposure and represent powerful tools that have the potential offer important insights into the molecular mechanisms involved in the development of allergy. Toxicogenomic analyses also have the advantage of profiling the entire transcriptome such that novel markers and new insights into the mechanisms of respiratory allergy can be identified. Published studies have already indicated that chemical allergens provoke a number of changes in gene expression in various tissues including lymph nodes, skin and pulmonary tissue and these changes have offered preliminary insights into the molecular pathways that may lead to the development of respiratory allergy. However, as with all toxicological investigations, full interpretation and application of the data will require the appropriate consideration of temporal-, dose-, and tissue-dependent responses and a systematic evaluation including a robust experimental design with genomic endpoints anchored to traditional immunotoxicology endpoints. Ultimately, the increased molecular understanding may be leveraged to develop novel biomarkers to classify agents with potential to induce respiratory allergy as part of the hazard characterization process. This presentation will describe the opportunities that exist for the identification and discrimination of respiratory and contact allergens based on mapping of exposure induced changes in gene expression.

W 907 A MODIFIED LOCAL LYMPH NODE ASSAY FOR HAZARD IDENTIFICATION OF CHEMICAL RESPIRATORY ALLERGENS.

T. Yoshida. *Health Science, Asahikawa Medical University, Asahikawa, Hokkaido, Japan.*

The LLNA was developed as a method for the characterization of contact allergens using mice and several attempts had been continued for reducing animal and RI usage in GHS. However, it is well established that chemical respiratory allergens also test positive in this assay and exploiting this as an alternative strategy for hazard identification will be addressed. Evaluation of cytokine gene expression relating Th1/2 in the lymphocytes of regional lymph nodes is useful to identify respiratory allergy. Chemicals are applied to ears in original LLNA but this challenging pathway is differing from actual respiratory exposure. In this presentation, the methods with the application of chemicals to nasal cavities or inhalation and measuring proliferation of lymphocytes and evaluation of cytokine profiles in each regional lymph nodes; cervical or lung hilar based on same concepts to LLNA will be introduced.

W 908 SAFER PRODUCTS FOR A SUSTAINABLE WORLD: LINKING CHEMICAL DESIGN AND TOXICOLOGY.

H. Zenick[1] and P. Beattie[2]. [1]*National Health Environmental Effects Research Lab., U.S. EPA, Research Triangle Park, NC and* [2]*SciVera, Inc. and Arcalis Scientific, LLC, West Bloomfield, MI.*

There is no more greatly studied characteristic of molecules than their ability to exhibit biological activity and major industries, including pharmaceuticals and pesticides, are based on this science. Billions of dollars are spent to evaluate the risk of chemicals in the environment and billions more are to discover new chemicals that have beneficial biological effects. One of the goals of green chemistry first introduced by Paul Anastas and John Warner in 1998, is to reduce or eliminate the use and generation of hazardous substances throughout the design, manufacture, and use of chemical products. The principles of green chemistry and engineering are now being widely embraced beyond the traditional chemical and pharmaceutical industries, into formulators and manufacturers of consumer products. Information on the potential hazards of the substances that are incorporated into final products is needed in order to assess and design safer products and processes. Recent advances in understanding the mechanisms of toxicity, the development of in vitro high-throughput screening (HTS) assays as well as other predictive and in silico methods allow for rapid assessment and screening of many more chemicals than had been possible in the past using traditional whole animal models. With the reform of the Toxic Substances Control Act (TSCA) and other state and global regulations requiring toxicity information and assessment of safer alternatives on thousands of chemicals, it is imperative that these predictive toxicology methods be incorporated into the assessment paradigm. As new industrial chemicals are designed with green chemistry and engineering principles, these toxicology screening methods can be used to efficiently evaluate substances, minimizing potential adverse health effects both in the workplace and to the final consumer.

W 909 GREEN CHEMISTRY DONE RIGHT: A PARTNERSHIP BETWEEN CHEMICAL DESIGN AND TOXICOLOGY.

P. Anastas. *Office of Research and Development, U.S. E.P.A., Washington, DC.* Sponsor: H. Zenick.

Given the tens of thousands of chemicals in use in the US and the world in dramatically increasing volumes, there is a great need to proactively design molecules, processes and products to minimize environmental and health impacts. There is a pressing need to learn how to design molecules that are inherently less toxic. Manufacturing processes need to be designed to use the least toxic substances for the job and to minimize downstream waste. The design or formulation of end-products should take into account environmental and health impacts through their entire lifecycle. The field of green chemistry encompasses these ideas. This talk will introduce the major principles of green chemistry with a focus on the role of toxicology in advancing these tenets using tool such as high throughput screening and computational and informational technologies

W 910 IMPLEMENTING CALIFORNIA'S GREEN CHEMISTRY INITIATIVE: THE TOXICOLOGICAL, TECHNICAL, AND EDUCATIONAL QUESTIONS.

M. Schwarzman. *Centers for Occupational and Environmental Health and Green Chemistry, University of California Berkeley School of Public Health, Berkeley, CA.* Sponsor: P. Beattie.

California EPA's Green Chemistry Initiative, and the laws that it has spawned, aim to establish a comprehensive approach to chemicals policy and the basis for green chemistry innovation. Implementing these changes requires input to address a variety of scientific needs, including rigorous methods for: Identifying chemicals of concern; addressing data gaps; conducting alternatives assessments; and developing new green chemistry curricula. Dr. Schwarzman will provide an overview of the state's initiative and discuss the ways the University of California, Berkeley is assisting in addressing these technical questions.

W 911 PUTTING WORKERS' SAFETY AND HEALTH INTO GREEN CHEMISTRY.

P. A. Schulte. *Centers for Disease Control and Prevention, NIOSH, Cincinnati, OH.* Sponsor: H. Zenick.

Early attempts at reducing the environmental impact of chemical manufacturing may have resulted in enhanced risk to workers' safety and health. Eliminating or reducing the environmental hazards associated with a chemical often requires the introduction of new processes or the modification of preexisting systems. These changes range from the production of smaller volumes of a substance to the replacement of an existing chemical with a "safer" alternative. These changes can result in unforeseen consequences if the occupational health hazards and associated risks are not considered. This presentation will highlight the impact of the green chemistry movement on workers' safety and health, in addition to emphasizing the need to eliminate occupational health hazards through the application of the principles of Prevention through Design

W 912 DIFFERENTIAL TOXICITY CHARACTERIZATION OF GREEN ALTERNATIVE CHEMICALS.

R. Judson. *National Center for Computational Toxicology, U.S. E.P.A., Research Triangle Park, NC.*

The goals of the Green Chemistry paradigm include reduction of the total volume of chemical consumption in manufacturing and end use, the reduction in persistence of chemicals that do get used, and reduction of the ultimate toxicity burden of these chemicals to end users and the environment. Predicting the final, intrinsic toxicity of a potential green replacement chemical is difficult because of the need to understand many classes of toxicity (cancer, reproductive and developmental toxicity, etc.). However, advances in *in vitro* high throughput screening (HTS), combined with databases of legacy whole animal toxicity data now enable for researchers to construct predictive toxicity models. These models may be ideally suited to the prediction of differential toxicity between a currently used chemical and a candidate green alternative. This talk will discuss the use of HTS, structure-based models, high throughput pharmacokinetic methods and data mining techniques to allow for the prediction of differential toxicity among sets of related chemicals, and will describe how one can make tradeoffs when the selection of one of a pair of chemicals is not straight forward.

W 913 DESIGN FRAMEWORK FOR SAFER CHEMICALS – RECENT RESEARCH AND COMMERCIAL APPLICATIONS.

T. G. Osimitz. *Science Strategies Institute and SciVera, Inc., Charlottesville, VA.*

One of the goals of the Green Chemistry approach is to reduce the inherent toxicity or hazard of chemicals in commerce. Accomplishing a shift in the hazard profile of commercial chemicals requires an understanding of how to rationally design commercial chemicals with reduced toxicity and a systematic review of currently used chemicals. Toxicologists provide knowledge about the nature of toxic effects. Once primarily a descriptive science, relying to a large extent on whole animal toxicology studies, the field has developed an extensive understanding of many of the mechanisms by which chemicals can exert toxicity. Application of this knowledge has made it possible to develop correlations, equations, and models that relate chemical structure and properties to biological responses. This has led to the use of sophisticated *in-silico* predictive models and provides the basis for current work being pursued in the development of a comprehensive design strategy for safer chemicals. Examples of recent research aimed at providing such design rules will be presented. In addition, the role that various industries can play in driving the shift towards Green Chemistry will be discussed. This will include the strategies that the supply chain can systematically apply for the assessment and evaluation of existing and alternative chemistries.

W 914 USING MODE OF ACTION DATA TO GUIDE QUANTITATIVE CANCER RISK ASSESSMENT: A CASE STUDY OF HEXAVALENT CHROMIUM IN DRINKING WATER.

D. Proctor[1] and B. Meek[2]. [1]*ToxStrategies, Inc., Rancho Santa Margarita, CA and* [2]*University of Ottawa, Ottawa, ON, Canada.*

Current cancer risk assessment guidance recommends that mode-of-action (MOA) data be used to inform the qualitative and quantitative assessment of human health risk. However, the ideal MOA data rarely exist, and as a result there are few examples for which MOA information has been used quantitatively in risk assessment. A comprehensive MOA research program was recently undertaken for hexavalent chromium [Cr(VI)] to better understand the key events underlying the tumorigenic response observed in rodents exposed chronically to Cr(VI) in drinking water. In 2009, and at the direction of an independent science advisory board, MOA Framework guidance was used to identify data gaps and develop a research program that included a pharmacokinetic (PK) study to generate the data necessary to model tissue dose, dose-response and quantify interspecies variability, and a subchronic study in both rats and mice (including an interim 7 day time point), with evaluation of the toxicogenomics in target tissues, histopathology, biochemical measures of immune response and oxidative stress, analyses of DNA damage (8-OH-dG and Cr-DNA adducts), and in vivo mutation. The findings of these studies are used to identify and temporally sequence key events in the MOA and provide information for
extrapolation across species and doses. The use of these MOA and PK studies for Cr(VI) cancer risk assessment are discussed as an example case study for using MOA data to refine human health risk assessment.

W 915 NTP TOXICOLOGY AND CARCINOGENESIS STUDIES OF CHROMIUM.

M. D. Stout and M. J. Hooth. *NTP/NIEHS, Research Triangle Park, NC.*

Hexavalent chromium (Cr(VI)) is a contaminant of water and soil and a human lung carcinogen. Trivalent chromium (Cr(III)) is a proposed essential element and is ingested by humans in the diet and in supplements, such as chromium picolinate. Extracellular reduction of Cr(VI) to Cr(III), which occurs primarily in the stomach, is considered a mechanism of detoxification, while intracellular reduction is thought to be a mechanism of carcinogenicity. The NTP has recently completed oral toxicology and carcinogenesis studies of Cr(VI) and Cr(III) in male and female F344/N rats and B6C3F1 mice exposed for 2 years. Cr(VI) was administered in drinking water as sodium dichromate dihydrate (SDD) and Cr(III) was administered in feed as chromium picolinate monohydrate (CPM). SDD was clearly carcinogenic to male and female rats and mice, inducing squamous neoplasms of the oral cavity (oral mucosa and tongue) in rats and epithelial neoplasms of the small intestine in mice. In rats and mice, exposure to SDD also resulted in reduced mean body weights and water consumption (due at least in part to reduced palatability), toxicity to the erythron, and histiocytic cell infiltration in the duodenum, liver and mesenteric and pancreatic lymph nodes. In mice, increased diffuse epithelial hyperplasia of the duodenum and jejunum was also observed. Non-neoplastic lesions were not increased in the oral cavity in rats. In contrast, there was no definitive evidence of toxicity or carcinogenicity following exposure to CPM. As part of these studies, total chromium was measured in tissues and excreta of additional groups of male rats and female mice. The disposition of Cr(VI) was inferred by comparing total Cr concentrations resulting from similar external doses of Cr(VI) and Cr(III). Much higher Cr concentrations were observed in all tissues following exposure to Cr(VI), indicating that at least some of the Cr(VI) escaped gastric reduction and was distributed systemically. Following exposure to Cr(VI), the shapes of the total Cr dose-response curves in tissues were linear or supra-linear, indicating that gastric reduction was not saturated.

W 916 RESEARCH PLAN TO FILL DATA GAPS IN THE MODE OF ACTION FOR CANCER RISK ASSESSMENT OF HEXAVALENT CHROMIUM IN DRINKING WATER.

L. Haws[3], D. Proctor[1], C. Thompson[2] and M. Harris[2]. [1]*ToxStrategies, Inc., Rancho Santa Margarita, CA,* [2]*ToxStrategies, Inc., Houston, TX and* [3]*ToxStrategies, Inc., Austin, TX.*

Using the mode-of-action (MOA) frameworks proposed by the U.S. EPA and ILSI/IPCS, we outlined key events and their temporal sequencing in a proposed MOA for intestinal tumors in mice following ingestion of Cr(VI). Data gaps identified during this MOA analysis were used to inform the design of a 90-day drinking water study to better inform the MOA for the observed responses. Sequentially, the proposed key events are saturation of the reductive capacity of the upper gastrointestinal tract, absorption of Cr(VI) into the intestinal epithelium, oxidative stress and inflammation, cell proliferation, DNA modifications (direct and/or epigenetic), and mutagenesis. Given clear species differences in carcinogenic response, key data gaps include direct measures of species-specific pharmacokinetic determinants (e.g. reduction and uptake), direct evidence for oxidative stress and inflammation as contributors to cell proliferation, and the nature of DNA modifications. Additionally, it is not known whether DNA modification and mutation occur at lower doses and early time points during exposure to Cr(VI) in drinking water. In order to address these data gaps, rats and mice were exposed to Cr(VI) for 7 or 90 days, followed by evaluation of histopathology, toxicogenomics, oxidative and inflammatory responses, DNA damage, in vivo mutations, target tissue chromium concentrations, and rates and capacities of Cr(VI) reduction in gastric fluid. These in vivo measures were collected in target tissues of both species at doses carcinogenic to rodents, as well as at concentrations more environmentally relevant to humans. Comparisons between responsive and unresponsive species and tissues will be used to further inform the carcinogenic MOA and provide mechanistic and dosimetric information necessary for human health risk assessment.

W 917 TOXICOGENOMIC ANALYSIS OF CR(VI) EFFECTS ON INTESTINAL AND ORAL EPITHELIUM.

T. R. Zacharewski[1], A. K. Kopec[1], A. L. Forgacs[1], S. Kim[1], S. D. Grimes[2] and C. D. Hébert[2]. [1]*Biochemistry & Molecular Biology and Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [2]*Southern Research Institute, Birmingham, AL.*

Chronic administration of hexavalent chromium (CrVI)-containing sodium dichromate dihydrate (SDD) is linked to intestinal and oral cancer in rodents. In this study, the dose-dependent gene expression effects of SDD (0, 0.3, 4, 14, 60, 170 or 520 mg/L) in female B6C3F1 duodenal, jejunal and oral epithelial tissues were examined after 7 days of exposure in drinking water. 4x44 K Agilent two-color oligonucleotide arrays identified 6453, 4360 and 698 unique gene expression changes in duodenum, jejunum and oral mucosa, respectively (|fold change|>1.5, P1(t)>0.999). Robust dose-dependent differential gene expression was identified in duodenal and jejunal epithelium. Automated dose-response modeling of the microarray data identified 2491 duodenal, 965 jejunal but only 141 oral mucosa genes displaying a sigmoidal dose-response profile with ED50 values within the experimental dose range. Comparisons between duodenal and jejunal expression profiles showed significant overlap with duodenal differential gene expression responses exhibiting greater efficacy compared to jejunum. The median and interquartile ED50 range overlapped between duodenum and jejunum with greater than 70% of all sigmoidal ED50 values between 10 and 100 mg/L SDD. Complementary histopathology identified crypt hyperplasia, cytoplasmic vacuolization in the intestinal epithelium and histiocytic infiltration in the villous lamina propria, consistent with the differential expression of genes associated with cell cycle, lipid metabolism and immune response in the duodenum and jejunum. Cancer associated genes were also over-represented within intestinal differential gene expression, and to a lesser extent in the oral mucosa. Collectively, these results indicate that intestinal epithelium is highly responsive to CrVI and early gene expression signatures may mediate neoplastic changes observed with prolonged SDD exposure.

W 918 GENETIC TOXICITY OF HEXAVALENT CHROMIUM IN THE GASTROINTESTINAL TRACT FOLLOWING ORAL EXPOSURE.

T. J. O'Brien[1], H. Ding[1], C. D. Hébert[2], R. D. May[2] and S. R. Patierno[1]. [1]*Department of Pharmacology and Physiology, The George Washington University Medical Center, Washington, DC and* [2]*Southern Research Institute, Birmingham, AL.*

Occupational exposure to hexavalent chromium [Cr(VI))]-containing compounds has been shown to cause lung cancer. Cr(VI)-induced carcinogenesis has been proposed to involve direct modification of DNA by Cr, inflammation and alteration of cell signaling pathways. Recently Cr(VI) has been shown to induce tumors of the duodenum of rodents following drinking water exposure at concentrations exceeding 60 mg/L sodium dichromate dihydrate (SDD). Because some chromium species interact with DNA, it is thought that DNA damage and mutagenecity play a role in the mode of action (MOA). To investigate the genotoxicity of Cr(VI) in the GI tract of rodents, SDD at 0, 0.3, 4, 14, 60, 170, 520 mg/L was administered ad libitum for 7 or 90 days. Samples were analyzed for 8-Oxo-2'-deoxyguanosine (8-OHdG) at 90 days (10 animals/group), and Cr-DNA binding at 7 and 90 days (5 animals/group). For Cr-DNA binding, DNA was isolated from scrapings of the oral, duodenal and jejunal epithelium and analyzed by ICP/MS. 8-OHdG analysis was performed on whole tissue from the duodenum and oral cavity using an ELISA. 8-OHdG levels were 2-3 times higher in the duodenal epithelium versus oral mucosa; however, a concentration-dependent increase in oxidized DNA bases was not observed. Concentration-dependent increases in Cr-DNA binding were observed in oral, jejunal and duodenal epithelium at 7 and 90 days, with evidence of the accumulation of Cr-binding observed overtime in all tissues. Slightly higher, but not statistically significant, levels of Cr-DNA binding were observed in the jejunum, as compared to the duodenum. In addition, an apparent threshold seems to exist for Cr-DNA binding in all tissues examined. Because we also observed villous cytotoxicity histopathologically, these results suggest that cytotoxicity in the duodenum villi may contribute to Cr(VI) carcinogenicity through a wound-rehealing mechanism involving the crypt cells.

W 919 A FRAMEWORK FOR EVALUATING DATA IN SUPPORT OF A MUTAGENIC MOA.

R. Schoeny. *Office of Water, U.S. EPA, Washington, DC.* Sponsor: D. Proctor.

To support its 2005 Guidelines for Cancer Risk Assessment the U.S. EPA Risk Assessment Forum convened a technical panel to develop guidance in evaluating data for determination that a chemical carcinogen acts through a mutagenic mode of action (MOA). The panel proposed definitions for mutagenicity and genotoxicity. Mutagenicity is the induction of permanent, transmissible changes in the amount, chemical properties, or structure of the genetic material. Genotoxicity is a broader term encompassing any alterations to genetic material including those that are transient. The technical panel noted that there is no "default MOA" for carcinogenicity but that all such judgements are data intensive exercises. A framework for data evaluation was proposed. While acknowledging the iterative nature of risk assessment, the framework considers 4 steps: 1. assemble all relevant data; 2. evaluate the data against current acceptance and quality criteria; 3. judge weight of evidence that the chemical has mutagenic activity, and if so, what type(s) of mutational activity; 4. by applying the Cancer Guidelines MOA framework, assess the evidence as to whether mutation is an early key event in the induction of tumors. The technical panel further proposed a hierarchy of data preference in making the weight of evidence decisions. The ideal data are cancer-relevant oncogene or tumor suppressor gene mutations detected in the tumor target tissue following chemical exposure. However, these results are rarely available, and the risk assessor must rely on more standard test batteries such as those from OCSPP guideline studies (i.e., gene mutations, cytogenetic damage, DNA adducts and/or primary DNA damage). Several case studies have been published that use principles of the proposed framework. Chloroform has sufficient evidence that the MOA does not include mutagenicity; whereas cyclophosphamide has sufficient evidence of a mutagenic MOA (McCarroll et al. 2008); and CrVI has adequate, but not overwhelming evidence of a mutagenic MOA (McCarrroll et al. 2010). [The opinions in this abstract are those of the author and do not necessarily reflect policies of the U.S. EPA.]

W 920 PHARMACOKINETICS OF INGESTED CR(VI) IN RODENTS: EXTRAPOLATIONS TO TARGET TISSUE DOSE IN HUMANS.

S. Hays[1], L. Aylward[2] and C. Kirman[3]. [1]*Summit Toxicology, Inc., Lyons, Co.,* [2]*Summit Toxicology, Inc., Falls Church, VA and* [3]*Summit Toxicology, Inc., Orange Village, OH.*

The target tissues for carcinogenic effects following chronic oral exposure to Cr(VI) in drinking water are the oral cavity in rats, and the small intestines (duodenum and jejunum) in mice, for which a response gradient is readily apparent (duodenum>jejunum>ileum) suggesting that neoplastic and non-neoplastic effects are due to direct contact of Cr(VI) in the lumen of the small intestine rather than systemic absorption. The ability of gastric and other body fluids to reduce Cr(VI) to Cr(III) before absorption occurs is recognized as a detoxification process providing protection against Cr(VI) toxicity following ingestion. However, saturation of reductive capacity occurs at some level of exposure, and serves as a potential source of nonlinear toxicokinetics for Cr(VI) . A series of studies were conducted to develop PBPK models to estimate species-specific target tissue dose following Cr(VI) ingestion. These included an ex vivo study of Cr(VI) reduction kinetics (rate and capacity) using real rat, mouse and human gastric fluid, and measures of Cr and Fe tissue

concentrations in six tissues and blood following 90 days of drinking water exposure at six doses from 0.1 to 180 mg Cr(VI)/L to refine existing PBPK models with rates of extracellular reduction, absorption, distribution and excretion in rats and mice. Because Cr competes with Fe and causes microcytic anemia, clinical measures of iron status were also collected to evaluate the affects on Cr kinetics. Using these species-specific PBPK models, we (1) estimated target tissue dose, characterizing tissue concentration gradients in the small intestine; (2) extrapolated between species; and (3) extrapolated from the high doses of Cr(VI) that causes cancer in rodents to the low doses to which humans are typically exposed.

W 921 USE OF MODE OF ACTION AND PHARMACOKINETICS TO INFORM THE CANCER RISK ASSESSMENT OF INGESTED CR(VI): A CASE STUDY.

D. Proctor[1], L. Haws[2], C. Thompson[3] and M. Harris[3]. *[1]ToxStrategies, Inc., Rancho Santa Margarita, CA, [2]ToxStrategies, Inc., Austin, TX and [3]ToxStrategies, Inc., Houston, TX.*

We conducted a Mode of Action (MOA) analysis for intestinal tumors observed in mice following chronic exposure to Cr(VI) in drinking water. Data gaps in the MOA were used to design a 90-day drinking water study including the collection of histopathological, biochemical, toxicogenomic, and pharmacokinetic data, as well as measures of DNA modification in rats and mice at concentrations of sodium dichromate dihydrate (SDD) from 0 to 520 mg/L. These data were used to evaluate whether the MOA for intestinal tumors is initiated through mutagenic or nonmutagenic mechanisms, interspecies differences, and dose-response at environmentally-relevant exposures. After 90 days of exposure in mice, crypt hyperplasia and villous atrophy were observed in the duodenum at exposures ≥ 170 mg/L SDD. Cytoplasmic signs of toxicity and stress were observed at ≥ 60 mg/L SDD. Clear signs of cellular oxidative stress (8-isoprostane) were apparent at ≥ 60 mg/L SDD, whereas changes in redox (GSH/GSSG) were apparent starting at 4 mg/L SDD. Together, these data suggest graded, dose-dependent changes in cell physiology beginning with mild oxidation, followed by oxidative stress, cytoplasmic toxicity, villous blunting and regenerative cell proliferation. These findings indicate a point-of-contact response for Cr(VI) in the lumen with the epithelium of the small intestine, and target tissue response in the mouse at concentrations ≥ 4 mg/L SDD. Oxidative DNA damage was not significantly elevated in the duodenum, but preliminary data on chromium content in genomic DNA of intestinal epithelium suggests a similar dose-dependent increase in the duodenum and jejunum of mice. These data, along with an evaluation of in vivo mutations and gene expression in the duodenum and oral mucosa of mice, and corresponding information in the rat, were used to inform the MOA for rodent tumors. Toxicokinetic models were used to relate tissue responses to relevant internal dose metrics, model dose-response, and for interspecies extrapolation.

HH 922 LOOKING BACK 50 YEARS OF TOXICOLOGY TO LOOK FORWARD.

S. G. Gilbert[1] and J. G. Pounds[2]. *[1]INND, Seattle, WA and [2]Cell Biology & Biochemistry, Pacific Northwest National Laboratory, Richland, WA.*

Toxicology has contributed enormously to environmental and human health. During the past 50 years significant rules and regulations were enacted to protect and enhance human and ecological health, often following a serious toxicological event. Most notably, toxicological sciences have shaped drug development through the U.S. FDA and chemical use through the U.S. EPA. Workplace exposure and health issues are addressed by OSHA and NIOSH. There is increasing concern about low-dose effects on the developing organisms of not only humans but also animals. Our panel of experts will review how advances in toxicological sciences over the past 50 years have driven the regulatory, societal, and medical view of key toxicants. After examination of some key contributions to the field, we will reflect on how advances in the science of toxicology will shape the future environment regarding drug and chemical development and use.

HH 923 METALS 50 YEARS OF EXPLORATION.

B. Weiss. *Department of Environmental Medicine, University of Rochester, Rochester, NY.*

Metals are enormously useful and essential in our society but are not without hazards to health. Although we knew long ago of the potential health hazards of lead and mercury, only in the last 50 years have we begun to appreciate the subtle kinds of damage they might exert, particularly to the developing nervous system. More recently, we have also become aware of their contribution to the disorders of aging, such as Alzheimer's disease. Moreover, metals we had conceived of primarily as essential components of the diet, such as manganese, are now viewed as potential threats to health as well, underscoring the importance of exposure context. Because we can now build bridges between health indices and cellular and molecular mechanisms, we are in a better position to apply scientific understanding to how we gauge health risks and their translation into policy and regulation. Tracing the history of metal toxicology provides a compelling lesson in how toxicology intersects with our lives as well as an example of how scientific progress tends to proceed in unpredictable directions.

HH 924 HALOGENATED AROMATIC HYDROCARBONS (PCBS, PBB, DDT, DIOXIN).

D. L. Eaton. *Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

Recognition that some chemicals are highly persistent in the environment was dramatically articulated to the public with the publication of "Silent Spring" by Rachel Carson in 1962 - one year after the founding of SOT. Subsequent incidences such as the inadvertent introduction of a polybrominated biphenyl flame retardant into animal feed with resulting widespread human exposures, and an industrial accident in Seveso, Italy that resulted in relatively large release of TCDD into the environment, contributed to widespread public recognition of the potential hazards of halogenated aromatic hydrocarbons to public health. Indeed, PCBs and DDT are still routinely found in wildlife and human breast milk, a legacy of decades of previous use. Toxicologists were central to discovering the molecular mechanisms by which such persistent environmental pollutants elicited toxicological effects such as hormone receptor-dependent changes in gene expression. Understanding the mechanisms, as well as environmental fate and transport of these compounds, has facilitated development of less hazardous compounds, and improved the ability of regulatory agencies to protect public health through science-based regulatory actions.

HH 925 RISK ASSESSMENT AND BEYOND.

E. M. Faustman. *Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

Risk assessment in its purist form brings together information on exposure and hazard to make an informed judgment on potential for harm. But it was soon discovered that that it is difficult to separate from risk management, risk communication and ultimately policy decisions. New perspectives such as the precautionary principle have also begun to shape risk assessment and policy decisions. In 1976 TSCA was passed and is now being considered for major revision. How is, or has, toxicology shaped chemical policy reform and what will regulatory policy look over the next 50 years? Toxicology and Toxicologists have and will continue to play an essential role in risk assessment however as we move towards a reductionist approach for identification and characterization of hazards, their expertise in systems based approaches for looking at the potential for chemicals to cause adverse endpoints across doses and conditions will be in even greater demand. Toxicologist will need to use their multidisciplinary training to predict impacts within a living system, across species and across life stages and levels of biological observation for informing risk assessments in the 21st Century and this will require our expertise to be in more demand and emphasize our use and understanding of kinetics and dynamics of response. It will also require us to re-think and energize our sometimes strained relationships with epidemiologists and clinicians to inform public decisions. Evolution of risk assessment will provide us with even greater opportunities to work with our colleagues in public health and exposure sciences.

HH 926 CHEMICAL CARCINOGENESIS 50 YEARS TO WHERE.

C. C. Harris. *National Cancer Institute, National Institute of Health, Bethesda, MD.* Sponsor: S. Gilbert.

The Delaney Clause, a 1958 amendment to the Food, Drugs, and Cosmetic Act stated in part that "the Secretary of the Food and Drug Administration shall not approve for use in food any chemical additive found to induce cancer in man, or, after tests, found to induce cancer in animals." The past 50 years have seen an unprecedented effort by toxicologist and the medical profession to understand the causes of environmentally-induced cancer. Toxicology has defined the role of metabolism, DNA damage and repair, inflammation, mutagenesis, and molecular/cell biology in individuals and populations at risk for cancer.

RI 927 A BRIEF INTRODUCTION TO UXO IN THE CAPITAL REGION: SESSION OVERVIEW.

E. R. Janus[2] and L. E. Roszell[1]. [1]*Environmental Health Risk Assessment, USA PHC (P), Aberdeen Proving Ground, MD and* [2]*Regulatory & Industry Affairs, Steptoe & Johnson LLP, Washington, DC.*

Unexploded Ordinance (UXO) are a product of military activities during the World War I and World War II eras. In addition to posing a serious threat from detonation, they also can contain chemical weapons such as arsenical compounds (Lewisite), sulfur mustard, and nerve agents. The discovery of bombs in our backyard poses significant challenges to characterization and remediation, outreach and risk communication, and inter-governmental relations. In order to examine this important issue, the history and issues posed by UXO in the U.S. Capital Region will be discussed with particular emphasis given to the former munitions experimental station located in the northwest Washington, D.C. neighborhood of Spring Valley. This station is a large, active World War I chemical munitions legacy site on the campus of the American University Experimental Station that was discovered during a trench excavation project in 1993. Since then, there have multiple phases of characterization and remediation activity, as well as health effects investigations conducted by both The Johns Hopkins University and the Agency for Toxic Substances and Disease Registry, a part of the Centers for Disease Control and Prevention.

RI 928 ENVIRONMENTAL RISK ISSUES ASSOCIATED WITH A MUNITIONS RESPONSE SITE IN NORTHWEST WASHINGTON, DC THE SPRING VALLEY FORMERLY USED DEFENSE SITE.

D. G. Noble. *Baltimore District, U.S. Army Corps of Engineers, Baltimore, MD.* Sponsor: L. Roszell.

The presenter, project manager for the site, will present one part of a three part discussion on this site to highlight the unique issues associated with munitions and the real and perceived risks associated with such items when they have been discarded in the environment. A brief, historical overview of the site will be provided, focusing on the American University Experiment Station – a World War I era facility that was the birthplace of the U.S. chemical warfare program. Munitions, especially chemical munitions, present unique challenges in investigating their potential locations at the site, assessing potential risks, safe recovery of the items, and eventual disposal. Interagency coordination, community outreach, and effective public relations are all important aspects of the project that often drive investigation and perception of risk in addition to the very real need to assess and determine risk derived from the potential chemical contamination left behind from testing activities involving military munitions. The presentation will be general in nature; but will highlight several very technical aspects of the project.

RI 929 MUNITIONS AND EXPLOSIVES OF CONCERN (MEC) IN SPRING VALLEY AND THE WASHINGTON DC AREA.

P. Greene. *USACE, Baltimore, MD.* Sponsor: L. Roszell.

Presentation will define Muntions and Explosives of Concern (MEC) and discuss types of MEC recovered in the Washington DC area with an emphasis on the Spring Valley location. Discussion of MEC will include specific hazards and precautions and impact of the environment on items which may have been buried for numerous years.

RI 930 TOXICITY OF WWI ERA CHEMICALS STUDIED AT SPRING VALLEY, WASHINGTON D.C.

C. Opdyke. *Baltimore District, U.S. Army Corps of Engineers, Baltimore, MD.* Sponsor: L. Roszell.

The Spring Valley Formerly Used Defense Site consists of approximately 661 acres in the northwest section of Washington, DC. During the World War I era, the site was known as the American University Experiment Station (AUES), and was used by the U.S. government for research and testing of chemical agents, equipment and munitions. Today, the Spring Valley neighborhood encompasses approximately 1,200 private homes, including several embassies and foreign properties, as well as the American University and Wesley Seminary. AUES studied the properties and efficiency of toxic substances - mustard gas, phosgene,superpalite (trichloromethyl chloroformate) - already in use in Europe. It suggested, developed, and submitted for testing many new chemical and solid toxic substances for possible use in gas warfare, among them, cyanogen chloride and bromobenzl cyanide. The AUES also investigated and developed smoke mixtures for Navy smoke screens and colored smokes for Army signaling on the battlefield, as well as incendiary material for use in bombs, shells, projectiles, darts and hand grenades. It conducted extensive tests related to the toxicity and symptomology of various classes of mustard gas and similar compounds. Many other toxic materials were studied and methods investigated for lethal delivery during wartime such as: adamsite, chloroacetic anhydride, nitrosomethylurethane, bromobenzl cyanide, thiophosgene, sulfur monochloride, cyanogen bromide, zinc arsenide and lewisite. The toxicity of such substances and the history of why these compounds were being studied at the AUES will be discussed and examined.

RI 931 A PUBLIC HEALTH APPROACH TO A WW I LEGACY: TRACKING HEALTH AND ENVIRONMENT IN SPRING VALLEY.

M. A. Fox[1], F. Curriero[2], R. Chari[1], E. Janus[4], K. Kulbicki[3], R. Neff[2], J. Zablotsky[5], B. Resnick[1] and T. Burke[1]. [1]*Health Policy and Management, Johns Hopkins University, Baltimore, MD,* [2]*Environmental Health Sciences, Johns Hopkins University, Baltimore, MD,* [3]*Westat, Rockville, MD,* [4]*Steptoe and Johnson, Washington, DC and* [5]*Food Safety and Inspection Service, U.S. Department of Agriculture, Washington, DC.*

From 1917 to 1918, the War Department conducted chemical warfare research on the American University campus and surrounding areas. Agents tested included arsenicals, phosgene, nitrogen and sulfur mustard gasses, among others. Testing activities caused wide dispersal of arsenic in soil and waste disposal resulted in localized subsurface contamination. An environmental health scoping study was conducted to: (1) Describe Spring Valley's community health status; (2) Identify health risks of past and present activities; (3) Identify the data gaps in the existing health and environmental information; and (4) Recommend health, exposure or environmental monitoring studies to fill remaining gaps. Four analyses were conducted: assessment community health status through vital statistic and registry data; identification of hazards; spatial analysis of exposure and outcomes; and health risk assessment and characterization including possible mixture exposures and cumulative risks. Community health in Spring Valley was good. Rates of major causes of mortality and incidence of selected cancers were 20% to 70% lower than the nation's. Rates of hypertension and related kidney disease mortality and certain potentially arsenic-related cancers were slightly elevated in Spring Valley over a comparison community. Registry-confirmed cancers were not more likely to cluster in higher contamination areas, Odds Ratio (95% Confidence Interval) = 0.60 (0.30, 1.11). Chemicals detected in disposal pits were associated with cancer, kidney, neurological and blood effects. In context of a healthy community, the elevated rates of certain mortality and cancer outcomes warrant continued monitoring. Follow-up on kidney, neurological and blood diseases was recommended. Further study is likely; the challenges of follow-up will be discussed.

RI 932 THE ROLE OF TRUST AT MUNITIONS/CHEMICAL WEAPONS SITES.

L. Siegel. *Center for Public Environmental Oversight, Mountain View, CA.* Sponsor: L. Roszell.

The investigation and remediation of the American University Experiment Station in Washington, DC's Spring Valley neighborhood is one of the country's highest profile formerly used defense sites (FUDS), but like other FUDS the project has operated under the shadow of inadequate appropriations. Furthermore, the Defense Department does not have clear policy for addressing sites - active, BRAC (Base Realignment and Closure), offshore, and FUDS - where chemical warfare materiel may have been discarded. Consequently, for many years critics of the Spring Valley project have questioned its sufficiency, particularly when the Army Corps declared victory and went home. This has created a situation in which many active community members mistrust the Army Corps and its regulators.

RI 933 ADDRESSING PUBLIC PERCEPTIONS: COMMUNICATING PUBLIC HEALTH ISSUES EFFECTIVELY.

L. S. Geckle. *Health Risk Communication program, U.S. Army Public Health Center (Prov), Aberdeen Proving Ground, MD.* Sponsor: L. Roszell.

Effectively addressing the public's concerns about health risks, whether real or perceived, involves a unique skill set that includes technical understanding, emotional intelligence, issues management, and crisis and emergency risk communication. This session will discuss risk communication considerations related to buried munitions found unexpectedly within the NCR. Lessons learned include the important but limited value of information-sharing; criticality of highly trained spokespersons; team support for a collaborative investigation process; and integration of academically based risk communication principles.

PL 934 THE FDA'S LIVER TOXICITY KNOWLEDGE BASE (LTKB) PROJECT.

W. Tong[1], M. Chen[1], V. Vikrant[1], Q. Shi[1], M. Zhang[1], L. Guo[2], Z. Liu[1], J. Zhang[1] and E. Bearden[1]. [1]*Division of Systems Biology, U.S. FDA's NCTR, Jefferson, AR and* [2]*Division of Biochemical Toxicology, U.S. FDA's NCTR, Jefferson, AR.*

A significant amount of drugs to be withdrawn from the market and to fail during clinical trial stage of development is due to liver toxicity. These cases of drug-induced liver injury (DILI) are reported as the leading cause of acute liver failure in the U.S. Thus, DILI has become one of the most important concerns in the drug development and approval process. DILI has also been identified by the FDA Critical Path Initiatives as a key area of focus in a concerted effort to broaden the agency's knowledge for better evaluation tools and safety biomarkers. In order to understand liver toxicity at the mechanistic level and develop novel tools for identifying liver toxicity issues along the various stages of drug development, there is a need to develop content-rich resources to improve our basic understanding of liver toxicity and facilitate the efficient development of novel knowledge and tools for utilization by research, industry and regulatory groups. The FDA's National Center for Toxicological Research is developing a liver toxicity knowledge base (LTKB). The project is drug-specific with aim to identify potential risk factors distinguishing drugs in DILI. Thus, the project involves a collection of diverse data (e.g., DILI mechanisms, drug metabolism, histopathology, therapeutic use, targets, side effects, etc) associated with individual drugs and systems biology analysis to integrate these data for DILI assessment and prediction. Importantly, both conventional and high-throughput molecular biomarker assays will be conducted for selected drugs to develop novel biomarkers based on knowledge accumulated from the project. The project will provide a wealth of focused knowledge and data mining tools for hepatotoxicity in the form of networks between chemicals (and drugs), molecular signatures, liver specific biomarkers, genes/proteins functions, pathways and liver diseases (and pathology). The preliminary results and early progress of LTKB will be discussed.

PL 935 VALIDATION OF CELLCIPHR™ CELLULAR SYSTEMS BIOLOGY (CSB) TECHNOLOGY. CELLCIPHR2™ - BUILDING ON EXPERIENCE OF *IN VIVO* RAT LIVER INJURY PREDICTION TO DEVELOP THE HUMAN DILI PREDICTION.

S. Thomas[2], J. Mein[2], R. Annand[1], P. Walker[2], M. Jacewicz[1], D. Steen[3], C. Chesne[3], J. Gilbert[1, 2] and K. Tsaioun[1, 2]. [1]*Apredica, Watertown, MA,* [2]*Cyprotex, Macclesfield, United Kingdom and* [3]*Biopredic, Rennes, France.*

Drug-induced liver injury (DILI) is the most frequent reason for the withdrawal of an approved drug from the market, and it also accounts for up to 50% of cases of acute liver failure. Nominating pre-clinical candidates is one of the most important activities in drug development, that clears the path for a compound to be tested in human clinical trials. Hence, it is of crucial importance for drug development teams to reduce the failures in preclinical toxicity studies. CellCiphr™ Cellular Systems Biology (CSB) technology is an in vitro discovery toxicology platform that has been validated by top large pharmaceutical companies and EPA, FDA, and NIH for identifying the risks of rat liver toxicity in new chemical entities. The most common uses of this technology currently are for lead series selection, lead optimization, and animal toxicity studies optimization. CellCiphr™ is built on high-content imaging and currently only predicts rat liver injury. As part of building of the human DILI prediction model, a sub-set of drugs with documented human DILI or lack thereof has been assessed in a new model in a number of rat and human transformed and primary liver models including HepG2, HepaRG and primary cells. Comparison of these models and their advantages and limitations in building classifiers for human prediction models will be presented for the first time. The utility of rat liver classifiers for prediction of human DILI will be assessed and new classifiers presented. CellCiphr technology is a mission-critical tool in drug development, and addition of human DILI model to already validated rat model is a significant advanced new tool allowing drug discovery teams to save significant funds by reducing the late attrition.

PL 936 INVESTIGATING THE ROLE OF MITOCHONDRIAL TOXICITY IN DRUG-INDUCED LIVER INJURY *VIA* CONGRUENT STRUCTURE-ACTIVITY RELATIONSHIPS.

M. L. Patel[1], L. Fisk[1], N. Greene[2] and R. T. Naven[2]. [1]*Lhasa Limited, Leeds, United Kingdom and* [2]*Worldwide Medicinal Chemistry, Pfizer Inc., Groton, CT.*

Drug-induced mitochondrial dysfunction may represent a key mechanistic pathway in the development of organ-related toxicities, including those affecting the liver. The silent accumulation of damage to mitochondria over time has been proposed as an explanation for observed idiosyncratic toxicity. As part of ongoing work to develop structure-activity relationships (SARs) to predict the toxicity of chemicals, we investigated the suitability of using existing hepatotoxicity structural alerts, as indicators of mitochondrial dysfunction. We report the evaluation of a hepatotoxicity knowledge base, using three published data sets for mitochondrial dysfunction.
Two data sets, comprising of 105 and 282 compounds, respectively, were constructed based on publications reporting in vivo results for mitochondrial toxicity. The third data set of 2490 compounds was based on the results of in vitro assays. The three data sets were processed against a knowledge base of 62 SARs for hepatotoxicity. Predictions were classed as correct when a hepatotoxicity alert was activated for a compound reported to cause mitochondrial dysfunction, or, in the absence of any alerts for a compound, reported to be negative. The results showed an overall concordance of 55% to 65% across the three data sets, with sensitivity ranging from 28% to 62% and specificity from 67% to 72%.
We have previously developed alerts for hepatotoxicity based on published in vivo data. A subset of these alerts performed well against the data sets reporting in vivo evaluations, indicating that for those chemical classes, the mechanisms of toxicity may act through the mitochondria. This was in contrast to the performance against the data set reporting in vitro results. Reasons for this could include the biased coverage of chemical space of the hepatotoxicity alerts, and the extrapolation of alerts based on in vivo data to predict in an in vitro scenario.

PL 937 QUANTITATIVE SIMULATION OF INTRACELLULAR SIGNALING CASCADES IN A VIRTUAL LIVER: ESTIMATING DOSE DEPENDENT CHANGES IN HEPATOCELLULAR PROLIFERATION AND APOPTOSIS.

J. Jack[1], M. Mennecozzi[2], C. Haugh[1], J. Wambaugh[1] and I. Shah[1]. [1]*National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC and* [2]*Joint Research Centre, European Commission, Ispra, Italy.*

The US EPA Virtual Liver (v-Liver™) is developing an approach to predict dose-dependent hepatotoxicity as an in vivo tissue level response using in vitro data. The v-Liver accomplishes this using an in silico agent-based systems model that dynamically integrates environmental exposure, microdosimetry, and individual cellular responses to simulate the in vivo hepatic lobule. One requirement for the v-Liver models is inclusion of nuclear receptor (NR) pathways critical to nongenotoxic hepatocarcinogenesis. Progress to date has focused on signal transduction networks controlling cell proliferation and apoptosis, which are key events in hepatocarcinogenesis. First, a literature derived knowledgebase was used to reconstruct a biochemical signaling network of cell cycle initiation and caspase-mediated apoptotic signaling events. The network contains 46 proteins and 77 interactions, encompassing pathways relevant to hepatocytes including the epidermal growth factor (EGF) and tumor necrosis factor alpha (TNFα). Second, static analysis of the topology of the crosstalk network revealed key signaling proteins, reducing the overall complexity of the model. Third, the mechanistic model was translated into an asynchronous Boolean network ensemble to simulate the quantitative dose-response of hepatocyte populations to various ligands: growth factors, cytokines, and mitogenic inhibitors. The model predictions are consistent with the synergistic effects of EGF and TNFα on early cell proliferation reported in the literature. Finally, the model was calibrated with ToxCast™ data on environmental compounds, revealing the potential impact of chemical perturbations on normal hepatocyte behavior. This approach of static and dynamic pathway analysis and evaluation using in vitro data provides a foundation for estimating tissue level effects of chemical exposures from the simulation of individual cellular responses. This abstract does not necessarily reflect US EPA policy.

PL 938 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING TO PREDICT PLASMA PHARMACOKINETICS FOR LIVER TRANSPORTER SUBSTRATES.

H. A. Barton[1], H. Jones[2] and Y. Lai[1]. [1]*Pharmacokinetics, Pharmacodynamics, and Metabolism, Pfizer, Inc., Groton, CT and* [2]*Pharmacokinetics, Pharmacodynamics, and Metabolism, Pfizer, Inc., Sandwich, United Kingdom.*

Predictions of plasma pharmacokinetics for substrates of liver uptake and biliary excretion transporters can be made using PBPK models. We have implemented a PBPK model for several drugs including fluvastatin, valsartan, rosuvastatin, and others. The well stirred liver is replaced with liver blood and tissue compartments connected by bidirectional passive diffusion and active uptake transport, while active efflux from liver to blood is assumed not to occur. Liver disposition includes nonspecific binding (partitioning), metabolism, and active biliary transport. Active transport processes, assumed to be occurring at concentrations below half maximal activity, are described by first order processes parameterized as clearances of free

concentrations in plasma or liver tissue. This two compartment model was compared with a 10-compartment model composed of 5 sequential blood compartments each connected to a liver tissue compartment. Gor some compounds the two models gave very similar results, but differences were observed for compounds with high active uptake. The multicompartment liver model is an approximation for the dispersion or tube models. The majority of the analyses have been for published human intravenous plasma profiles, but modeling for the rat has been completed for a more limited set of compounds. In addition to modeling the in vivo data to obtain estimates for the various processes, in vitro data from human liver microsomes and sandwich cultured hepatocytes have been used. Comparisons of predictions for plasma concentration profiles based upon in vitro results have been made to estimate in vitro to in vivo extrapolation factors. The in vitro to in vivo extrapolation factors vary for the different processes being scaled. The analyses demonstrate the value of capturing mechanistic biological processes in pharmacokinetic modeling to improve the ability to predict pharmacokinetic disposition.

PL 939 A NOVEL PREDICTIVE PLATFORM FOR DILI: A COMBINATION OF *IN SILICO* AND *IN VITRO* METHODS TO PREDICT TOXICITY MECHANISMS *IN VIVO.*

S. Das, R. Kumar, S. Raghavan and K. Subramanian. *Strand Life Sciences, Bangalore, India.*

Prediction of hepatotoxicity is still a challenge in the drug development process. Currently available methods are inadequate both for extrapolation of rodent information to humans as well as in predicting the likelihood of idiosyncratic liver injury. Species used in preclinical toxicity do not usually possess underlying aberrations leading to idiosyncratic toxicity observed in humans in the clinic.
We have developed a dynamic systems model of the rat liver to predict DILI. The metabolic network modeled integrates the biology behind different types of drug induced toxicity. Important biological processes involved in fat metabolism (steatosis), energy homeostasis (necrosis), oxidative stress (oxidative damage) are modeled quantitatively. A set of differential equations predict changes in metabolite concentrations The model was first validated by comparing model predictions under conditions of normal liver homeostasis and DILI with observations published in the literature. We then used the model to generate hypotheses for the mechanism behind idiosyncratic toxicity caused by the anti-diabetic drug troglitazone.
Sensitivity analysis of the metabolic network identified a set of enzymes that are predicted to be key players in causing different types of DILI. In vitro measurements of these enzyme rates, using a primary hepatocyte system, in absence and presence of well-known hepatotoxic drugs, are used as input to the model. Model simulated outputs of toxicity for each drug matched well with the associated toxic phenotype reported in the literature. The validation of this combinatorial (in silico and in vitro) approach using known hepatotoxic drugs e.g. Diclofenac, Cyclosporin, Tamoxifen, etc. yielded additional novel mechanistic insights.
The purpose of study is to develop a generalized liver toxicity prediction platform integrating in vitro and in silico approaches. Such an approach allows us to predict toxicity, generate mechanistic insights, and mechanism specific biomarkers.

PL 940 APPLICATIONS OF A COMPUTATIONAL MODEL FOR PREDICTING DRUG INDUCED LIVER INJURY (DILI).

B. A. Howell[1], R. Kumar[3], L. Wennerberg[3], Y. Yang[2], R. Ho[3], A. Harrill[1], C. L. Kurtz[1], M. E. Andersen[2], H. J. Clewell[2], S. Q. Siler[3] and P. B. Watkins[1]. [1]*Drug Safety Sciences, The Hamner Institutes, Research Triangle Park, NC,* [2]*Chemical Safety Sciences, The Hamner Institutes, Research Triangle Park, NC and* [3]*Entelos, Inc., Foster City, CA.*

Drug-induced liver injury (DILI) is the adverse drug event that most frequently leads to termination of clinical development programs and regulatory actions on drugs. We have developed a predictive model based on physiological processes involved in DILI, focusing initially on acetaminophen toxicity. A physiologically based pharmacokinetic (PBPK) model was developed to describe the disposition and metabolism of APAP. Reactive metabolite production and subsequent covalent protein binding are characterized. Glutathione (GSH) depletion and synthesis, oxidative stress, mitochondrial dysfunction, and the cell life cycle, including regeneration and death, are modeled. The model accurately reproduced APAP pharmacokinetic measures and GSH depletion/recovery for rats, mice, and humans, and this explained much of the variation in species specific susceptibility to APAP induced DILI. In-depth comparisons were also made between rats and mice with regard to their ability to re-synthesize GSH after GSH depletion. The use of N-acetyl-cysteine (NAC) as a treatment for APAP overdose was incorporated into the model, and treatment times were analyzed and compared to standard clinical practices to predict optimal NAC use. Finally, the model was used to successfully predict the species differences in hepatotoxicity of methapyrilene using in vitro to in vivo extrapolation. The absorption, distribution, and metabolism of methapyrilene were estimated from the physical characteristics of the compound and in vitro metabolism. The model correctly predicted that rats would be less sensitive to acetaminophen than mice or humans, but most sensitive to methapyrilene. A parametric search for in silico rats, mice, and humans susceptible to methapyrilene DILI predicted that methapyrilene would cause very little hepatotoxicity in humans clinically. This finding agrees with clinical reports.

PL 941 DEVELOPMENT OF A PBPK/PD MODEL FOR ACETAMINOPHEN TOXICITY IN RATS.

Y. Yang, B. Howell, M. Yoon, M. Andersen and H. J. Clewell. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

A whole-body physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model for acetaminophen (APAP) and its metabolites in the rat was developed to quantitatively describe the processes that determine APAP hepatotoxicity. The key features of the model include metabolic activation of APAP by cytochrome P450s, detoxication of APAP via sulfate and glucuronide conjugation, and binding of the reactive metabolite N-acethyl-p-benzoquinone imine (NAPQI) to hepatic glutathione (GSH) and cellular macromolecules. Both sulfate and glucuronide conjugation was described using Michaelis Menten equations. Sulfation was modeled as a high affinity, low capacity pathway, while glucuronidation was described as a low affinity, high capacity pathway. A description of the depletion of the cofactor for sulfation (PAPS) and its precursor inorganic sulfate was required to properly simulate APAP-sulfate concentrations in the rat blood. Enterohepatic recirculation of the conjugates was also included. The net production of NAPQI is determined by the balance between the bioactivation via cytochrome P450s and the detoxification by conjugation with GSH. The model also describes the resulting depletion and recovery of GSH by incorporating a feed-back regulation of GSH synthesis. The model successfully simulated concentration-time profiles of APAP, sulfate and glucuronide conjugates of APAP after oral, intraperitoneal, and intravenous (iv) administrations ranging from 37.5 to 1000 mg/kg doses. The dose-dependent changes in hepatic GSH concentrations after IV dose were also well described. This model will contribute to better understanding of the mechanism of APAP hepatotoxicity by providing a tool for predicting the time-course of reactive metabolite formation and glutathione depletion in studies measuring hepatic damage and repair.

PL 942 USING ZEBRAFISH TO STUDY BENZO(A)PYRENE-MEDIATED CHANGES IN DNA METHYLATION STATUS DURING DEVELOPMENT.

X. Fang[1], C. Thornton[1], B. E. Scheffler[2] and K. L. Willett[1]. [1]*Department of Pharmacology and Toxicology, the University of Mississippi, University, MS and* [2]*Genomics and Bioinformatics Research Unit, USDA Mid South Area Genomics Laboratory, Stoneville, MS.*

DNA methylation is one of the epigenetic mechanisms to control gene expression and is vulnerable to *in utero* or early-life environmental toxicant exposures. Constitutively, we confirmed that zygote demethylation is conserved in zebrafish and characterized dynamic CpG methylation patterns in 5 genes (vasa, Ras-association domain family member 1 (RASSF1), telomerase reverse transcriptase (TERT), c-Myc and c-Jun). Direct waterborne benzo(a)pyrene (BaP) treatment at 100 μg/L from ~2 to 96 hours post fertilization (hpf) to zebrafish embryos significantly decreased global cytosine 5' methylation by 55% and gene-specifically reduced promoter CpG methylation in vasa by 17.2%. Future study will measure the vasa mRNA expression to determine correlated altered expression. However, BaP did not change CpG island methylation in RASSF1, TERT, c-Myc and c-Jun at 96 hpf. To study the mechanisms of demethylaion, mRNA expression and enzyme activity of 2 methylation related enzymes, DNA methyltransferase 1 (DNMT1) and glycine N-methyltransferase (GNMT), were measured constitutively and after treatment. BaP exposure at 1, 10 or 100 μg/L did not change DNMT1 and GNMT mRNA expression at 48, 60 or 96 hpf or nuclear total DNMT and cytosolic GNMT enzyme activity at 96 hpf. In summary, BaP is a potential epigenetic modifier for global and gene specific DNA methylation status in zebrafish embryos and the alteration is possibly not mediated by affecting DNMT1 and GNMT expression. (Support: NIEHS R01ES012710).

PL 943 GENOME-WIDE DNA METHYLATION DIFFERENCES BETWEEN LATE-ONSET ALZHEIMER'S DISEASE AND COGNITIVELY NORMAL CONTROLS IN THE HUMAN FRONTAL CORTEX.

K. Bakulski[1], D. C. Dolinoy[1], M. A. Sartor[1], H. L. Paulson[2], A. P. Lieberman[2], R. L. Albin[2], H. Hu[1, 2] and L. S. Rozek[2]. *[1]School of Public Health, University of Michigan, Ann Arbor, MI and [2]Medical School, University of Michigan, Ann Arbor, MI.*

Sporadic cases of late-onset Alzheimer's disease (LOAD) are the most globally prevalent form of dementia, resulting in significant public health and economic burdens. Discordant twin studies highlight the non-Mendelian characteristics of LOAD and prompt a further look at its potential environmental etiology. Primate studies demonstrate early life exposure to the heavy metal lead influences brain β-amyloid deposition, a major hallmark of Alzheimer's disease (AD), as well as DNA methyltransferase activity in late life. This foundational human study investigates DNA methylation differences between LOAD cases and controls, so that future work may associate environmental exposures with LOAD-specific epigenetic change. In 24 human post-mortem frontal cortex tissues from LOAD and cognitively normal age- and gender-matched subjects, quantitative DNA methylation was measured at 27,578 CpG sites spanning 14,475 genes via the Illumina Infinium HumanMethylation27 BeadArray. Based on parallel linear models with empirical Bayes methods, 958 CpG sites representing 928 unique genes were significantly associated with disease status. The top gene hit was recently implicated in Amyloid Precursor Protein post-translational processing, supporting a role for epigenetic change in AD pathology. Over 3,500 CpG sites were associated with an age-specific epigenetic drift, highlighting the importance of age adjustment in statistical modeling of disease methylation in late life. Future studies will evaluate cumulative lead exposure, as determined by ICP-MS lead levels in the occipital bone, to investigate the association between lead and LOAD-specific epigenetic change as a potential toxicological mechanism of disease. In human, relevant LOAD-disease tissue, this study demonstrates significant discordant DNA methylation and invites additional research associating environmental exposures.

PL 944 EPIGENETIC CHANGES DURING CO-CARCINOGENESIS OF N, N-DIETHYLNITROSAMINE AND CARBON TETRACHLORIDE IN MOUSE LIVER.

K. Kutanzi[1], I. Koturbash[1], T. Uehara[2], O. Kosyk[2], I. Pogribny[1] and I. Rusyn[2]. *[1]National Center for Toxicological Research, U.S. FDA, Jefferson, AR and [2]Department of Environmental Sciences and Engineering, UNC, Chapel Hill, NC.*

Global genomic hypomethylation and transcriptional silencing of cancer-related genes induced by promoter CpG island hypermethylation are important epigenetic events during human and rodent hepatocarcinogenesis. In human hepatocellular carcinoma, which develops mostly under conditions of liver fibrosis and cirrhosis, both global DNA methylation levels and gene-specific methylation status in tumorous and adjacent non-tumorous tissues have been established as important for tumor development and predictors of recurrence and overall patient survival. In animal models of experimental chemical-induced liver carcinogenesis, the role of fibrosis and epigenetic alterations is less understood. This study aimed to examine the epigenetic alterations caused by fibrosis and fibrosis-promoted tumor development in the mouse liver. Male B6C3F1 mice were treated with either a single dose (1 mg/kg) of N,N-diethylnitrosamine (DEN) or PBS at 15 days of age. Carbon tetrachloride (CCl4) treatment (0.2 ml/kg; i.p.; 2/week) was started at 8 weeks of age and continued for 9 or 14 weeks. CCl4 treatment led to a severe bridging fibrosis with nodule-like features, which was accompanied by liver inflammation and steatosis. DEN treatment resulted in few histopathological changes except for the formation of pre-neoplastic foci. Loss of global DNA methylation and methylation of the repetitive DNA elements (i.e., LINE-1) in non-tumorous liver tissue was observed in CCl4 and CCL4+DEN (22 weeks of age) groups. At the same time, methylation-specific PCR showed progressive hyper-methylation of the promoters of p16(Ink4a), Rassf1a and Mgmt genes. This study provides evidence that progressive liver fibrosis is not only acting as a tumor promoter after DEN-initiation, but also has an effect on epigenetic modifications of the DNA which may also play a role in the mechanism of chemical-induced liver carcinogenesis in the mouse.

PL 945 AH RECEPTOR INHIBITS GENE EXPRESSION OF DELETED IN LIVER CANCER (DLC1) THROUGH REGULATION OF MICRORNA IN MOUSE LIVER CELLS.

Y. Tian[1], S. Ke[1], M. Huang[2], B. Zhou[1] and Z. Zhou[2]. *[1]Program of Toxicology, Texas A&M University, College Station, TX and [2]School of Public Health, Fudan University, Shanghai, China.*

Dioxin and related compounds have been shown to promote hepatocarcinogenesis in rodent. Although the mechanism for the tumor promotion activity of dioxin is not clear, the activity is dependent on the aryl hydrocarbon receptor (AhR). MicroRNA, together with other non-protein coding RNA have recently been found to possess critical functions for physiological and pathophysiological processes including carcinogenesis. In order to understand the mechanism of cancer promotion by dioxin we analyzed microRNA profiles in the mouse hepatocytes exposed to dioxin (10 nM, 24 hr). By surveying the microRNA regulated by AhR, we have identified a novel AhR target DLC1 (deleted in liver cancer 1) whose expression was regulated at post-transcriptional level by AhR complex. We found that activation of AhR by TCDD significantly suppressed the mRNA of DLC1 which is critical for hepatocarcinogenesis. The inhibition of DLC1 expression by dioxin is mediated by the AhR complex as both AhR and the AhR partner protein ARNT were required for the suppression of DLC1 by dioxin. The 3'UTR of DLC1 harbors several target sites of dioxin-induced microRNA. The reporter gene directed by the 3' UTR of DLC1 is responsive to dioxin treatment in a AhR-dependent manner. These results collectively suggest that DLC1 is an important target for ligand-activated AhR and AhR-regulated DLC1 gene expression may be important for the liver cancer promoting activity of dioxin and related toxic compounds (Supported in part by ES 09859 from NIEHS).

PL 946 INTER-STRAIN DIFFERENCES IN SUSCEPTIBILITY TO 1, 3-BUTADIENE-INDUCED DNA DAMAGE, EPIGENETIC AFFECTS AND HEPATOTOXICITY.

I. Pogribny[1], K. Igor[1], A. Scherhag[1], J. Sorrentino[2], S. Kennet[2], W. Bodnar[2], S. A. James[2], B. A. Frederick[1] and R. Ivan[2]. *[1]National Center for Toxicological Research, Jefferson, AR and [2]University of North Carolina, Chapel Hill, NC.*

1,3-Butadiene (BD) is a major industrial chemical and a common environmental contaminant which is classified as carcinogenic to humans. Both genetic and epigenetic factors may influence individual susceptibility to environmental toxicants and it has been suggested that BD exposure may yield variable adverse health outcomes in humans. In order to model the inter-individual differences in genetic and epigenetic effects of BD, we used a panel of inbred mouse strains (NOD/LtJ, CAST/EiJ, A/J, WSB/EiJ, PWK/PhJ, C57BL/6J and 129S/SvImJ) exposed to 0 or 625 ppm BD for 6 hours/day, 5 days/week for 2 weeks. The goals of this study were to compare the magnitude of inter-individual differences in BD toxicity between individual inbred strains of mice, and to investigate possible mechanisms of the effects on DNA damage, epigenetic alterations and hepatotoxicity. In CAST/EiJ strain, liver levels of N-7-(2,3,4-trihydroxybut-1-yl)guanine adducts in DNA were about 2-fold lower than in other strains. Epigenetic effects were evident at the level of both DNA and histones. Global loss of hepatic DNA methylation was observed in PWK/PhJ, C57BL/6J, and 129S/SvImJ mice, with the greatest effect being detected in C57BL/6J mice. Loss of trimethylation of H3 lysines 9 and 27, and H4 lysine 20 was found in C57BL/6J mice, while the opposite effect was detected in CAST/EiJ mice. Interestingly, gene expression analysis demonstrated altered expression of genes that are indicators of liver toxicity in C57BL/6J mice only. These findings demonstrate that significant epigenetic events occur following BD and suggest that the differences in inter-individual susceptibility to BD exposure may be determined by variations in epigenetic response.

PL 947 ALTERED DNA METHYLATION PATTERNS IN INDIVIDUALS WITH ARSENICOSIS.

L. Smeester[1], J. Rager[1], L. Zhang[6], X. Guan[3], N. Smith[1], G. Garcia-Vargas[4], L. Del Razo[5], Z. Drobna[2], H. Kelkar[3], G. Schroth[6], M. Styblo[2] and R. Fry[1]. *[1]Environmental Sciences and Engineering, Gillings School of Global Public Health UNC-Chapel Hill, Chapel Hill, NC, [2]Nutrition, Gillings School of Global Public Health UNC-Chapel Hill, Chapel Hill, NC, [3]Center for Bioinformatics, UNC-Chapel Hill, Chapel Hill, NC, [4]Faculty of Medicine, Juarez University of Durango State, Juarez, Mexico, [5]Department of Toxicology, CINVESTAV-IPN, Mexico City, Mexico and [6]Illumina, Inc., Hayward, CA.*

Inorganic arsenic (iAs) is a documented carcinogen and an environmental toxicant, poisoning tens of millions of people worldwide through drinking water. Individuals exposed chronically to iAs are susceptible to arsenicosis, or arsenic poisoning. Such

exposure is associated with the development of skin lesions and in some cases skin cancer. One of the proposed mechanisms of iAs-induced disease is altered gene regulation via epigenetic modes of action such as DNA methylation. Here we set out to identify differentially methylated genomic regions associated with arsenicosis in humans exposed to iAs via their drinking water. Lymphocyte DNA of sixteen individuals from the arsenicosis endemic region of Zimapan, Mexico was analyzed. Of these individuals, half showed signs of arsenicosis with skin lesions. The methylomes of two individuals selected with or without skin lesions were first compared at single nucleotide resolution using Reduced Representation Bisulfite Sequencing. All samples were subsequently analyzed using the Methylated CpG Island Recovery-Chip assay. These combined analyses assess differential methylation at more than 1 million CpG sites within ~14, 000 CpG islands. Mapping CpG sites to islands and performing comparative analysis resulted in the identification of genes with differential methylation patterns associated with arsenicosis. These genes are enriched for their involvement in cancer-associated pathways such as p53 and NF-kB. These data demonstrate the significant effects of iAs on the epigenome and suggest that changes in DNA methylation patterns may serve as biomarkers of adverse health effects associated with iAs exposure.

PL 948 HDAC INHIBITORS, ENTINOSTAT AND VORINOSTAT, SYNERGIZE WITH ADAPHOSTIN, A TYRPHOSTIN KINASE INHIBITOR, INCREASING OXIDATIVE STRESS AND APOPTOSIS IN LEUKEMIA CELLS.

N. Rivera-Del Valle and J. Chandra. *Pediatric Research, University of Texas MD Anderson Cancer Center, Houston, TX.*

The major mechanism of action for histone deacetylase inhibitors (HDACi) is through hyperacetylation of histones. Interestingly, several studies have indicated that HDACi cause oxidative stress, which contributes to the cytotoxicity of these drugs. Combining agents that cause further oxidative stress might enhance the efficacy of HDACi for the treatment of leukemia. Adaphostin is one such agent. Studies have shown that adaphostin increases levels of reactive oxygen species (ROS). The objective of this study was to investigate the possible synergy of HDACi and adaphostin in leukemia cells. Jurkat cells were exposed to different concentrations of adaphostin and entinostat or vorinostat. After 24 hr, DNA fragmentation was assessed by propidium iodide staining followed by flow cytometric analysis. A combination index (CI) less than 1.0 is representative of synergism as measured by Calcusyn software. Results showed a synergistic effect on DNA fragmentation when combining the 2.5 μM of entinostat with 750 nM of adaphostin (CI=0.6). The same effect was observed when combining 500 nM adaphostin with 500 nM vorinostat (CI=0.67). This synergistic effect was blocked when cells were pre-treated with 50 μM of the pan-caspase inhibitor ZVAD or 24 mM of the antioxidant N-acetylcysteine. These results suggest that the mechanism involved in this synergism is caspase and ROS dependent. To test the involvement of oxidative stress, we measured superoxide levels by hydroethidium staining. Significant increases in superoxide levels were observed with the combination of adaphostin with entinostat or vorinostat ($p<0.05$), confirming that oxidative stress plays an important role in the synergism observed. Future studies will be directed to determine the mechanism by which HDACi and adaphostin induce oxidative stress mediated-apoptosis. Understanding the mechanism involved in this synergism will advance scientific knowledge of how the action of HDACi could be augmented in leukemia models.

PL 949 AHR-MEDIATED IMMUNE REGULATION RESULTS FROM EPIGENETIC EFFECTS.

P. Nagarkatti, N. P. Singh, U. Singh and M. Nagarkatti. *Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC.*

Aryl hydrocarbon receptor (AhR) is a member of a transcription factor superfamily. Recent studies have suggested that AhR activation leads to differential regulation of T cells. To address this further, we compared the effects of various AhR ligands on T cell differentiation and epigenetic modulation. To this end, we used TCDD, 6-formylindolo[3,2-b]carbazole (FICZ), and indigoids (Indirubin, I3C, and DIM), with different affinities for AhR. Culture of murine T cells activated through the T cell receptor in the presence of various AhR ligands led to different degrees of T cell differentiation. For example, there was a significant increase in Tregs in the presence of TCDD, Indirubin, I3C, and DIM, whereas in the presence of FICZ, there was a significant increase in the generation of Th17 cells but not of Tregs. The same differentiation pattern was observed using antigen-specific T cells. Upon examination of epigenetic effects of various AhR ligands on Foxp3 and IL-17 gene promoters, we observed significant demethylation of Foxp3 promoter but methylation of IL-17 promoter in the presence of TCDD, Indirubin, and I3C. However, FICZ was observed to have completely different epigenetic effects on the promoters of these two genes. Furthermore, we also examined the effects of AhR ligands on micro RNAs (miRNAs) profile in T cells. There was a significant difference in the profiles of various miRNAs in T cells treated with various AhR ligands. Together, the data from these studies demonstrate that AhR ligands can have differential effects on T cell differentiation into Th17/Tregs and that such an effect may be regulated by epigenetic modulation (Supported in part by NIH grants R01ES09098, P01AT003961, R01ES019313).

PL 950 DNA METHYLATION AND HISTONE MODIFICATION CHANGES IN THE LIVERS OF C57BL/6J MICE EXPOSED TO 1, 3-BUTADIENE BY INHALATION.

I. Koturbash[1], A. Scherhag[1], S. Jessica[2], B. Wanda[2], S. A. James[2], B. A. Frederick[1], P. Igor[1] and R. Ivan[2]. [1]*Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR and* [2]*University of North Carolina, Chapel Hill, NC.*

The need for rapid determination of potential human carcinogens is one of the priority tasks for modern toxicology. Traditionally, research emphasis in assessment of chemical exposure has focused on genetic alterations and was limited to detection of DNA damage, DNA adduct formation, and mutations induced by specific agents in target tissues. However, recognition of the role of epigenetic alterations as one of the underlying mechanisms in carcinogenesis has challenged this traditional approach to carcinogenicity testing. 1,3-Butadiene (BD) is a well-known toxic agent with carcinogenic activity in both humans and rodents that is largely attributed to genotoxicity of its reactive intermediates. The aim of the preset study was to determine if BD may also be epi-genotoxic (i.e. cause changes in DNA and histone methylation) and to explore the possible molecular mechanisms for the epigenetic changes. Short term exposure of C57BL/6J mice to BD by inhalation (625 ppm; 6 hours/day; 10 days) led to the formation of N-7-(2,3,4-trihydroxybut-1-yl)guanine adducts in hepatic DNA and, in addition, caused extensive epigenetic alterations. The epigenetic abnormalities included demethylation of global DNA and repetitive elements, and a decrease in histone H3 and H4 lysine methylation. In addition, there was down-regulation of maintenance DNA methyltransferase Dnmt1 and histone methyltransferase Suv39h1, and up-regulation of the demethylase Jmjd2a, which are proteins responsible for the accurate maintenance of the epigenetic marks. These findings demonstrate that exposure to BD leads to epigenetic alterations in the liver, which may be important contributors to the mode of BD carcinogenicity.

PL 951 PROPICONAZOLE ENHANCES CELL PROLIFERATION BY DYSREGULATION OF RAS FARNESYLATION AND THE MAPK PATHWAY.

L. Murphy, T. Moore and S. Nesnow. *U.S. EPA, Research Triangle Park, NC.*
Sponsor: S. Hester.

Previous studies of mice exposed to the hepatotumorigenic fungicide, propiconazole, revealed an increase in hepatic cell proliferation and over-expression of hepatic genes within the cholesterol biosynthesis pathway. Mevalonate, an intermediate in this pathway, has long been a target for anti-cancer therapies as mevalonate promotes proliferation in numerous cancer cell lines and serves as the precursor for isoprenoids (e.g. farnesyl pyrophosphate) which are crucial in the farnesylation of the Ras protein. Farnesylation targets Ras to the cell membrane where it is involved in signal transduction of numerous pathways, including the mitogen-activated protein kinase (MAPK) pathway. The goal of this study was to determine if mevalonate and propiconazole would increase AML12 hepatocyte proliferation by modulation of Ras membrane localization and further downstream effects. Mevalonolactone (MVAL) treatment increased cell numbers, while treatment with L-744,832, a farnesyl transferase inhibitor, reversed the MVAL mediated increase in proliferation. AML12 cells treated with propiconazole led to a significant ($p<0.05$) increase in proliferation which was reversed by co-incubation with L-744,832. Western analysis of membrane and cytoplasmic fractions from control and MVAL or propiconazole treated cells reveal increased Ras protein in the membrane fraction of MVAL and propiconazole treated cells as well as a reduction in the cytoplasmic fraction. This effect was reversed when cells were exposed to either MVAL or propiconazole and L-744,832. Preliminary western analysis indicates that phosphorylation of Erk1/2, a MAPK protein downstream of Ras, can be modulated by propiconazole or MVAL. Taken together, these results indicate that propiconazole regulates Ras

membrane localization and subsequent signal transduction via modulation of Ras farnesylation. This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

PL 952 ANALYSIS OF MOLECULAR, CELLULAR, AND BIOCHEMICAL CHANGES IN THE LIVER OF MALE B6C3F1 MICE TREATED WITH NITRAPYRIN—A NITROGEN STABILIZER.

M. J. LeBaron[1], M. R. Schisler[1], H. L. Kan[1], J. Thomas[1], D. L. Eisenbrandt[2] and B. B. Gollapudi[1]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Dow AgroSciences, Indianapolis, IN.*

The purpose of this study was to investigate potential modes-of-action (MoA) contributing to nitrapyrin (2-chloro-6-(trichloromethyl)pyridine)-induced hepatocellular tumors in B6C3F1 mice. In this MoA study male B6C3F1 mice were fed 0, 75, 250, or 400 mg nitrapyrin/kg body weight/day (mkd) for 7 or 14 days. Another group was given nitrapyrin for 14 days then switched to a control diet for 21 days to investigate recovery. Body weight decreased slightly in animals given 400 mkd with treatment-related decreases in serum cholesterol at 250 and 400 mkd after 7 or 14 days of treatment, which were within normal limits after the recovery period. Mice given 250 or 400 mkd nitrapyrin for 7 or 14 days had increases in liver weights, consistent with the histopathologic findings of centrilobular/midzonal hypertrophy with cytoplasmic eosinophilia. Analysis of hepatocellular proliferation via BrdU incorporation indicated a clear dose- and hepatolobular zone-related induction of S-phase DNA synthesis. Recovery group animals had no treatment-related histopathologic liver alterations. Gene expression analysis of the liver indicated a direct, robust dose-related increase in the Cyp2b10/CAR-associated transcript with an associated increase in Cyp2b10 protein. The lack of associative enzyme activity suggested that nitrapyrin administration resulted in mechanism-based (suicide) inhibition of the enzyme. Gene expression analysis indicated no activation of AhR, PXR, or PPAR-α signaling pathways. In summary, nitrapyrin-induced effects were consistent with the causal key events related to CAR-mediated rodent liver tumorigenesis and were completely reversible upon treatment cessation. Moreover, these data provided convincing evidence that key events and hepatocellular tumors do not occur at or below 75 mkd. A Human Relevance Framework evaluation supports that this phenobarbital-like MoA for mouse liver tumors associated with high doses of nitrapyrin is not relevant to humans.

PL 953 THE FRY TUMOR SUPPRESSOR ENCODES AN INHIBITOR OF EPITHELIAL MESENCHYMAL TRANSITION.

J. Graham[1], X. Ren[2] and H. Zarbl[1]. [1]*Toxicology, UMDNJ-RWJMS, Piscataway, NJ and* [2]*UC, Berkeley, CA.*

We previously identified Fry, the rat ortholog of the Drosophila furry gene, as a Mammary Carcinoma Susceptibility locus. FRY is reduced > 40% in human breast cancer cell lines relative to the nontransformed MCF10A mammary epithelial cell line, suggesting that decreased FRY may contribute to carcinogenesis. To evaluate the function of the Fry gene, we stably transfected the triple-negative, human MDA-MB-231 mammary tumor cell line with the wild type (wt) allele from Cop (231wCFry). In Matrigel, the 231wCFry cells formed polarized mammospheres resembling those formed by the nontumorigenic MCF10A cells, while parental MDA-MB-231 cells formed disorganized clusters. When injected into nude mice, tumors formed by the 231wCFry cells were 8-fold smaller, encapsulated and non-invasive in sharp contrast to MDA-MB-231. Gene-expression profiling revealed that Fry regulates gene networks that maintain cellular and tissue organization, differentiation, motility and proliferation. The Wnt/β-catenin signaling pathway, which maintains cell polarization, was most significantly changed by Fry. Immunocytochemistry revealed that the levels and subcellular distribution of β-catenin and integrins in 231wCFry, both markers of epithelial cell differentiation, more approximated those in MCF10A than in MDA-MB-231, suggesting Fry restored a differentiated phenotype to the cancer cells in vitro. Consistent with a role in suppression of tumor progression, analysis of microarray data for 2,200 human breast tumor samples (Oncomine 3.0 Cancer Profiling Database) revealed that FRY was decreased in aggressive breast cancers. In summary, ectopic expression of wt Cop Fry in tumor cells enhanced cell polarization, adhesion, and differentiation, while suppressing tumor growth and invasiveness. These observations suggest that Fry expression can reverse or suppress epithelial mesenchymal transition (EMT), a process involved in the acquisition of invasive and metastatic phenotypes, making FRY a significant new target for therapeutic interventions.
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PL 954 REDUCTION OF PTEN DOSE IMPAIRS DNA REPAIR AND PREDISPOSES TO UVB-INDUCED SKIN TUMORIGENESIS.

Y. He[1], M. Ming[1], L. Feng[2], C. Shea[1], K. Soltani[1], B. Zhao[2], W. Han[1], R. Smart[3], C. Trempus[2] and J. Pritchard[2]. [1]*Medicine/Dermatology, University of Chicago, Chicago, IL,* [2]*Laboratory of Pharmacology, National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC and* [3]*Department of Environmental and Molecular ToxicologyGroup, North Carolina State University, Raleigh, NC.*

Non-melanoma skin cancer is the most common cancer in the US. The major environmental risk factor causing skin cancer is the UVB radiation in sunlight through damaging DNA. The tumor suppressor PTEN, a critical regulator for multiple cellular processes, is frequently mutated or deleted in many human cancers. Here we show that target reduction of PTEN levels in epidermal keratinocytes is a predisposing factor for UVB-induced skin tumorigenesis in mice. In contrast to the response to ionizing radiation, PTEN down-regulation prolongs UVB-induced arrest of growth and activation of the DNA damage checkpoint Chk1 pathway. PTEN down-regulation impairs the capacity of global genomic nucleotide excision repair (GG-NER), a critical mechanism for removing UVB-induced mutagenic DNA lesions. At the molecular level, PTEN loss suppresses the expression of the key GG-NER protein xeroderma pigmentosum C (XPC) through the AKT/p38 axis. Reconstitution of the XPC levels in PTEN-inhibited cells restores the GG-NER capacity. In human skin neoplasia, PTEN is significantly down-regulated in both premalignant actinic keratosis and malignant squamous cell carcinoma, suggesting a critical role of PTEN in both tumor development and progression. Therefore, these findings demonstrated that PTEN is an essential genomic gatekeeper in the skin through positively regulating XPC-dependent GG-NER following UVB damage.

PL 955 TOLFENAMIC ACID INHIBITS GROWTH AND SURVIVAL OF MULTIPLE TUMORS AND DOWNREGULATES SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS.

G. Chadalapaka[1], I. Jutooru[1], S. Sreevalsan[1], S. Pathi[1] and H. Wilson[2]. [1]*VTPP, Texas A&M University, College Station, TX and* [2]*Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX.*

Cancer is the second leading cause of death in United States. In spite of improvements in conventional anticancer therapies, the number of cancer deaths hasn't been significantly reduced. Current research in our laboratory focuses on the anticancer activity and toxicology of tolfenamic acid, a mechanism-based non-steroidal anti-inflammatory drug (NSAID) that targets downregulation of specificity protein (Sp) transcription factors. RNA interference studies support the vital role of specificity protein (Sp) transcription factors as an important target for anticancer agents since Sp1, Sp3 and Sp4 proteins are overexpressed in most tumors and regulate expression of genes required for cancer cell survival, metastasis (survivin, bcl-2, NFkB), growth (cyclin D1, epidermal growth factor receptor-EGFR, c-MET) and angiogenesis (vascular endothelial growth factor [VEGF]) and their receptors. Tolfenamic acid targets downregulation of Sp proteins in pancreatic, colon and esophageal cancer cells and the effects were investigated in SJCRH30 and RD human rhabdomyosarcoma cancer cells and a series of canine melanoma, mammary cancer and osteosarcoma cells. In this study, tolfenamic acid (50-200 μM) significantly inhibited growth of rhabdomyosarcoma and different canine cancer cell lines; western blot analysis showed that Sp1, Sp3 and Sp4 proteins were overexpressed in these cells and treatment with 50-200 μM Tolfenamic acid decreased expression of Sp1, Sp3, Sp4 and several Sp-regulated gene products including VEGF, EGFR, p65 (NFkB), c-Met and survivin. These results demonstrate that tolfenamic acid, a drug currently in use for treating acute and chronic pain, may also be an important new anticancer compound for treatment of human and canine cancers.

PL 956 ROLE OF SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS IN REGULATION OF STAT3 IN PANCREATIC CANCER CELLS.

I. D. Jutooru[1], G. Chadalapaka[1] and S. H. Safe[1, 2]. [1]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX and* [2]*Institute of Biosciences & Technology, Houston, TX.*

Signal Transducer and Activator of Trancription (STAT) is activated by various cytokines and growth factors resulting in dimerization and phosphorylation of STAT's. Activated STAT binds to specific DNA-response elements and promotes cell growth, development, inflammation and immune responses. Constitutive activation of STAT3 is detected in most cancer types including pancreatic cancer and

plays an important role in survival, drug resistance and angiogenesis. The human STAT3 promoter region has several cis-elements including CRE/ATF, SBE and GC boxes. Studies in this laboratory showed that Sp proteins (Sp1, Sp3 & Sp4) that bind GC-rich sequences are overexpressed in many tumor types and are associated with poor prognosis in pancreatic cancer patients. Since Sp proteins also regulate genes associated with survival, angiogenesis and growth, we investigated the possible interactions between Sp proteins and STAT3 and genes regulated by these important trans-acting factors. Results from individual knockdown (RNA interference) of Sp1 (siSp1), Sp3 (siSp3), and Sp4 (siSp4) and combined knockdown Sp1, Sp3, and Sp4 (siSp) on expression of STAT3 were examined in Panc28 and L3.6pL cells. Transfection of Panc28 and L3.6pL cells with siSp1 or siSp4 decreased expression of pSTAT3 and STAT3 but siSp3 had no effect on STAT3 expression. However, the effect of combined knockdown (siSp) of all 3 Sp proteins was much more effective than individual Sp knockdown in both cell lines. In contrast, knockdown of STAT3 (siSTAT3) did not effect expression of Sp1, Sp3 or Sp4 however siSp decreased expression of several putative STAT3 regulated genes such as Cyclin D1, survivin and VEGF (vascular endothelial growth factor). Thus results of Sp1, Sp3, and Sp4 knockdown by RNA interference demonstrate that both pSTAT3 and STAT3 are Sp-regulated genes in pancreatic cancer cells suggesting that agents (betulinic acid, curcumin and celastrol) targeting Sp transcription factors will also decrease STAT3.

PL 957 PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR)γ IN MAMMARY SECRETORY EPITHELIAL CELLS STOPS DMBA-MEDIATED BREAST TUMOURIGENESIS.

A. J. Apostoli[1], N. Peterson[1], R. Rubino[1], S. SenGupta[1], M. Schneider[1] and C. Nicol[1, 2]. [1]*Pathology & Molecular Medicine, Queen's University, CRI, Kingston, ON, Canada and* [2]*Division of Cancer Biology & Genetics, Queen's University, CRI, Kingston, ON, Canada.*

One in every 28 women will die from the growth and spread of breast cancer. Our previous studies with peroxisome proliferator-activated receptor (PPAR)γ heterozygous mice suggest PPARγ normally suppresses 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast tumour progression, but the mechanisms and cell types involved remain unknown. Given the proposed transdifferentiation between mammary secretory epithelial (MSE) cells and adipocytes, we hypothesize that MSE-specific PPARγ signaling normally acts to prevent DMBA-mediated breast tumour progression. Here we generated *Cre*/loxP-based, MSE cell-specific PPARγ knockout (PPARγ-MSE KO) mice. Eight week old female PPARγ-MSE KO mice and their congenic wildtype (PPARγ-WT) controls were mated to induce pregnancy and allowed to lactate for 3 days to ensure complete PPARγ deletion in MSE cells. All lactating dams were then treated by gavage once/week for 6 weeks with 1 mg DMBA. At week 7, females were randomized to receive either a normal chow diet (DMBA Only: PPARγ-WT, n=10 and PPARγ-MSE KO, n=17) or one supplemented with a PPARγ activating drug (DMBA+ROSI: PPARγ-WT, n=12 and PPARγ-MSE KO, n=10) for the study duration (25 weeks). Our data suggests that DMBA Only-treated PPARγ-MSE KO mice had a decreased median survival (week 17 vs 23 respectively), a 2-fold decrease in mammary tumour latency ($p<0.05$), and larger mammary tumour volumes compared to respective controls. Among DMBA+ROSI-treated mice, median survival increased (PPARγ-MSE KO: 20 weeks and PPARγ-WT: >25 weeks) and mammary tumour volumes decreased (PPARγ-MSE KO: ~3-fold and PPARγ-WT: ~3-fold) compared to DMBA Only-treated mice. These results suggest that normal PPARγ signaling in MSE cells contributes to the suppression of DMBA-mediated breast tumourigenesis. They also add evidence supporting a protective role for PPARγ activation in stopping environmental chemical-mediated breast tumour progression.

PL 958 MITOCHONDIAL UNCOUPLING PROTEIN 3 ANTAGONIZES TUMOR PROMOTION BY ALTERING CANONICAL KERATINOCYTE GROWTH AND DIFFERENTIATION SIGNALING PATHWAYS.

S. M. Nowinski[1], J. E. Rundhaug[2], O. Rho[1], J. Digiovanni[1] and E. M. Mills[1]. [1]*Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX and* [2]*Science Park - Research Division, The University of Texas MD Anderson Cancer Center, Smithville, TX.*

Compared to normal tissues, nearly all malignancies exhibit increased glycolysis and decreased oxygen consumption. In contrast, mitochondrial uncoupling proteins drive futile mitochondrial respiration that is uncoupled from ATP synthesis. We previously showed that mice expressing a keratin 5 - uncoupling protein 3 (K5-UCP3) transgene in the epidermis are completely protected from the formation of skin carcinomas in response to a DMBA/TPA chemical carcinogenesis regimen. To explore the mechanism of UCP3 induced cancer resistance, our lab inter-bred K5-UCP3 animals with Tg.AC mice that express a cutaneous oncogenic v-Ha-Ras transgene. Whereas wild type Tg.AC mice form tumors in response to TPA treatment alone, K5-UCP3/Tg.AC animals are protected from TPA-induced tumorigenesis, indicating that UCP3 likely inhibits tumor promotion. The data support the hypothesis that UCP3 blocks promotion by increasing keratinocyte differentiation, resulting in the loss of tumorigenic cells through normal skin turnover. Indeed, K5-UCP3 mice exhibit increased skin turnover and increased markers of differentiation in vivo and in vitro. Rhod-2 staining revealed elevated cytoplasmic calcium in K5-UCP3 cells, which is a known important intermediate event in normal keratinocyte differentiation. Several signaling pathways implicated in keratinocyte differentiation are also augmented in K5-UCP3 cells, including Protein Kinase Cδ phosphorylation (Western Blotting) and Notch 3 signaling (q-PCR). Increased keratinocyte differentiation should correspond to growth arrest. Interestingly, Akt phosphorylation is modified in K5-UCP3 cells, while other growth pathways are largely unaffected. These data reveal novel roles for UCP3 in keratinocyte biology, and suggest that mitochondrial uncoupling is a promising new approach to cancer prevention.

PS 959 EFFECTS OF LOW LEVELS OF BISPHENOL A IN HUMAN ENDOTHELIAL CELLS.

H. Andersson and E. Brittebo. *Pharmaceutical Biosciences, Faculty of Pharmacy, Uppsala, Sweden.* Sponsor: M. Stigson.

Epidemiological evidence report an association between exposure to bisphenol A and cardiovascular disease and diabetes[1]. Alterations in endothelial cell functions play an important role in the development of cardiovascular disorders and diabetes and it is therefore important to study the cell specific effects of bisphenol A in this cell type.
The aim of the present study was to investigate the effects of low levels of bisphenol A in human umbilical vein endothelial cells (HUVEC). The role of nuclear and cytosolic receptors previously associated with the effects of bisphenol A was also investigated. HUVEC were treated with 0.1-1000 nM bisphenol A for 6 or 24 hours and markers of endothelial dysfunction, inflammation and angiogenesis where assayed using qRT-PCR, western blot and fluorometric assays measuring nitric oxide and reactive oxygen species. The results revealed that 6 hours incubation of HUVEC with low dose bisphenol A increased the production of nitric oxide (10 nM; $P<0.01$, 1000 nM; $P<0.05$), increased mRNA expression of eNOS (1-1000 nM; $P<0.05$) and increased the protein expression of phosphorylated eNOS (1000 nM) bisphenol. An increase in VEGFA mRNA expression was observed following 6 hour's of exposure to 1 nM ($P<0.05$), 10 nM ($P<0.01$) and 1000 nM ($P<0.05$) bisphenol A and also after 24 hour's of exposure to 10 nM ($P<0.001$) bisphenol A. Nitric oxide and VEGFA function as hyperpermeability factors that promote vascular permeability and may contribute to endothelial dysfunction . These preliminary results suggest that bisphenol A can induce changes in human endothelial cells previously known to be important for vascular function at levels equivalent to levels of human exposure.
[1] Melzer et.al., Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06. PLoS One. 2010 Jan 13;5(1)

PS 960 CIGARETTE SMOKE EXPOSED PRECISION-CUT LUNG SLICES (PCLS) AS A NEW *IN VITRO* MODEL FOR SMOKE-INDUCED TOXICITY.

S. Switalla, D. Ritter, J. Knebel, C. Dasenbrock, A. Braun and K. Sewald. *Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany.* Sponsor: H. Muhle.

Introduction: Chronic obstructive pulmonary disease (COPD) is a severe lung disease with high mortality and increasing prevalence. It is characterized by profound damage of lung parenchyma due to cigarette smoke-induced oxidative stress. To understand the underlying mechanisms the need emerged to develop human relevant models. Lipopolysaccharide (LPS) is a widely used model compound which does not directly induce oxidative stress due to lacking radicals. It therefore can not reflect cigarette smoke-induced toxicity in the lung. The aim of the studies was to establish precision-cut lung slices (PCLS) as a relevant toxicity model by using cigarette smoke and cigarette condensate in comparison to LPS as model compounds.
Methods: Murine PCLS were prepared and exposed submerged to LPS and cigarette condensate, and to cigarette mainstream smoke using the PRIT® air-liquid interface (ALI) culturing and exposure system. Induced toxicity was assessed by vitality staining and determination of enzymatic activity using the WST-1. Pro-inflammatory immune responses connected to toxic and subtoxic doses were quantified using ELISA. Additionally, dexamethasone and roflumilast were applied in combination with model compounds.
Results: Exposure to cigarette condensate and cigarette smoke induced a clear toxicity for concentrations ranging from 37-300 μg/mL for cigarette condensate and exposure doses of 0.6 and 1.2 μg/cm^2 for cigarette smoke compared to their con-

trols, respectively. Subtoxic and toxic doses of cigarette condensate induced a significant release of tumor necrosis factor (TNF)-α and interleukin (IL)-1α. Release of TNF-α and IL-1α for 5 and 100 ng/mL of LPS was inhibited by dexamethasone and roflumilast.
Conclusion: PCLS represent a promising test system to reflect the toxic aspects of cigarette smoke-induced tissue damage occurring in COPD but it furthermore can be used to study pharmacological intervention.

PS 961 LONG-TERM CULTURE OF H9C2 RAT CARDIOMYOCYTES WITH NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTIS) ALTERS RESPIRATORY CAPACITY AND MITOCHONDRIAL BIOGENESIS.

Y. Liu, P. Nguyen, T. Baris and M. C. Poirier. *National Cancer Institute, Bethesda, MD.*

The nucleoside reverse transcriptase inhibitors (NRTIs), antiretroviral drugs used for therapy of HIV-1, have achieved long-term survival of patients with AIDS. However, mitochondrial dysfunction, considered due to effects on mitochondrial DNA replication, is a dose-limiting toxicity of these drugs, and progressive cardiac mitochondrial damage has been observed in mouse and monkey offspring exposed in utero to NRTIs. To elucidate underlying mechanisms, here we exposed H9c2 cells to no drug, 50 μM Zidovudine (AZT), and 50 μM AZT/50 μM Didanosine (ddI), continuously for 39 passages. Respiratory integrity was examined using the Seahorse XF24 analyzer to evaluate oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). These NRTI doses supported cell survival (by Incucyte) at all passages, with a moderate inhibition of cell growth at early passages (70-89% survival) and resistance after P13 (90-112% survival). The maximal OCR, determined in uncoupled cells, was 40-80% of control ($p<0.05$) for both treatment groups for most passages between P2 and P36. The corresponding EACR values were normal until P11, becoming significantly elevated ($p<0.05$) at P18-31. Evaluation of mitochondrial biogenesis (MitoBiogenesis Assay, MitoSciences), revealed a moderate reduction (~20%) at the early passages, an increase (~20%) after P11 and substantial depletion by P39 ($P<0.05$). This rat cardiomyocyte model will be useful for evaluation of mechanisms underlying NRTI cardiac toxicity, and for evaluation of strategies for intervention.

PS 962 INTERROGATING GENETIC PATHWAYS OF TOXICITY USING *IN VITRO* HIGH-CONTENT SCREENING OF EMBRYONIC FIBROBLASTS FROM THE MOUSE DIVERSITY PANEL.

T. Wiltshire[1], O. Suzuki[1], N. Butz[1], A. Frick[1], B. Parks[2], J. Trask[2] and R. Thomas[2]. [1]*University of North Carolina, Chapel Hill, NC and* [2]*The Hamner Institute for Health Sciences, Durham, NC.*

A key component of the vision laid out in the NRC report 'Toxicity Testing in the 21st Century' is the development of assays based on toxicity pathways. Toxicity pathways are defined as cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects. Despite the importance of these pathways, no strategy was put forth on how to identify them. In this presentation, we describe the use of primary embryo fibroblasts isolated from 32 genetically characterized inbred mouse strains to experimentally identify potential toxicity pathways. mouse embryo fibroblasts (MEFs) were exposed to one of a 75 of diverse environmental and pharmaceutical agents in dose response (9 concentrations; 17 nM to 100 uM) and for two different exposure intervals (24 and 72 hrs). The effects of exposure were assessed using high-content screening assays for integrated cellular responses that included cell permeability, nuclear count and morphology, mitochondrial membrane potential, and cytochrome C release. Phenotypic differences in sensitivity to the different treatments allowed genetic association mapping to be performed on each endpoint and time point, resulting in a collection of candidate loci that underlie these cellular responses. Candidate genes can then be tested for their contribution to mechanisms of toxicity by overexpression and siRNA knockdown experiments in the MEF cell lines. Validation of genes involved in Rotenone sensitivity have been completed. Enrichment analysis on these candidate loci identified a number of critical signaling pathways for each endpoint. For cell death, enriched processes included cell cycle transitions and critical nodes included known regulators such as Tp53. Aggregating the cellular processes and signaling pathway nodes across the diverse treatments, endpoints, and time points will provide a set of experimentally identified toxicity pathways that can be used to implement the 'Toxicity Testing in the 21st Century' vision.

PS 963 VEHICLE EFFECTS ON RESULTS OF LOCAL LYMPH NODE ASSAYS.

T. Anzai[1, 2], D. Hayashi[1, 2], S. Corney[1], K. Sato[2] and T. Satoh[3, 1]. [1]*Harlan Laboratories, Tokyo, Japan,* [2]*Showa University School of Medicine, Tokyo, Japan and* [3]*HAB Research Institute, Chiba City, Japan.*

The Local Lymph Node Assay (LLNA) is now regarded as the worldwide standard alternative method for identifying potential skin sensitizing test substances. The analysis of accumulated LLNA data reveals that the vehicles employed are likely to affect LLNA results. Here we show that a vehicle difference in the response was observed when CBA/CaHsdRcc mice were exposed to 6 vehicles; 4:1 v/v acetone/olive oil (AOO), ethanol/water (70% EtOH), N,N-dimethylformamide (DMF), 2-butanone (BN), propylene glycol (PG) and dimethylsulfoxide (DMSO). The dpm/LN level was lowest in the 70% EtOH group and highest in the DMSO group. When alpha-hexylcinnamaldehyde (HCA) was used as a sensitizer for the LLNA, HCA was a weak sensitizer when AOO or DMSO was used as a vehicle, but a moderate sensitizer when the other 4 vehicles were used. This study showed that there are vehicle differences in the local lymph node response (dpm/LN level) in the LLNA and that the sensitization potency of HCA may be classified in different categories when using different vehicles. This suggests that careful consideration should be exercised in selecting a vehicle for the LLNA.

PS 964 EFFECTS OF 5-HT_{1A} OR ENDOCANNABINOID RECEPTOR AGONISTS ON ZEBRAFISH BEHAVIOR IN NOVEL ENVIRONMENTS.

K. A. Connors[1], T. W. Vallenti[1], K. N. Prosser[1], B. W. Brooks[1], E. S. Onaivi[2] and G. G. Gould[3]. [1]*Environmental Science, Baylor University, Waco, TX,* [2]*Biology, William Paterson University, Wayne, NJ and* [3]*Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Acute behavioral tests for zebrafish (*Danio rerio*) are amenable for use in neurotoxicology because of their design efficiency. Parallels to mammalian limbic system circuits are present, and binding sites for mood-altering drugs, such as cannabinoid (CB) and serotonin (5-HT) receptors appear functionally conserved in teleost fish. This may allow for a read-across extrapolation between these species. We measured CB receptor density in the zebrafish brain using the agonist [^{3}H] WIN 55,212 at 10 nM, and found that it was 6.0±0.3 pm/mg protein (pr) in hypothalamus, and 7.3±0.3 pm/mg pr in dorsal telencephalon. 5-HT_{1A} receptor saturation and displacement binding by the agonist [^{3}H] 8-OH-DPAT in zebrafish whole brain homogenates revealed a maximal binding (Bmax) of 1200±336 fmol/mg pr and a dissociation constant (KD) of 23±3 nM (N=3). The 5-HT_{1A} agonist buspirone displaced [^{3}H] 8-OH-DPAT with a Ki =100±15 nM as compared to Ki=14±8 nM for WAY 100,635. To examine in vivo effects of 5-HT_{1A} and CB receptor agonists, we utilized two novel environment anxiety behavior tests, the dive tank and aquatic light-dark plus maze. In the plus maze following an acute 10 minute aquatic exposure to either buspirone (50 mg/L), or 1-week dietary exposure to WIN55,212 (1 μg/d), zebrafish spent more time in and/or entered white arms more often than controls ($p<0.05$, N=6-8). Buspirone at 25 mg/L increased top dwelling in the dive tank, whereas WIN55,212 did not. Acute 10 minute exposure to WIN55,212 at 0.5 mg/L, but not at higher doses reduced mobility in the plus maze, whereas 5 or 50 mg/L tended to increase time spent in white arms (N=9). As predicted from mammalian data, these compounds displayed anxiolytic properties in zebrafish placed in novel environments. Further, such behavioral responses appear useful for neurotoxicology modeling to examine adverse pathways as alternative endpoints during environmental risk assessment.

PS 965 IMPLEMENTATION OF THE NCTC2544 IL-18 ASSAY TO IDENTIFY *IN VITRO* CONTACT ALLERGENS.

V. Galbiati[1], M. Mitjans[2], F. Colleoni[1], C. L. Galli[1], M. Marinovich[1] and E. Corsini[1]. [1]*Department of Pharmacological Sciences, Università degli Studi di Milano, Milano, Italy and* [2]*Dpt Fisiologia, Facultat de Farmacia, Universitat de Barcelona, Barcelona, Spain.*

We previously reported the use intracellular interleukin-18 (IL-18) production in the human keratinocyte cell line NCTC2455 in assessing in vitro of the contact sensitization potential of low molecular weight chemicals. The purpose of this work was to implement this assay in order to: 1. to optimize experimental conditions to limit the cell density issue; 2. to develop a 96-well plate format to establish a high throughput assay; 3. to test the performance of other available keratinocyte cell lines; and 4. to understand the signal transduction pathway involved in p-phenylediamine (PPD)-induced IL-18 production.

The effect of cell density on the response to the contact allergen PPD was investigated. After over night adherence, 1 to 2.5 x 105 cells/ml represents the optimal cell density, higher cell density (> 5 x 105 cells/ml) resulted in a loss of effect. This problem can be overcome treating cells after 2 h of cell adherence.
A selective increase in total IL-18 was also observed exposing cells seeded in 96-well plate (1 to 2.5 x 104 cells/well) to a panel of contact allergens, whereas no changes were observed following treatment with respiratory allergens and irritants. Similar results were also obtained testing with PPD other keratinocyte cell lines, namely HPKII and HaCaT, with the HPKII showing the highest stimulation index. Regarding the signal transduction pathway, we could demonstrate using selective inhibitors, namely PDTC, Bay 11-7085 and SB 203580, a role for oxidative stress, NF-kB and p38 MAPK activation in PPD-induced IL-18 production.
Our data suggest that production of IL-18 indeed represents a promising endpoint for the screening of potential contact allergens. The assay can be performed in a 96-well plate format, different keratinocyte cell lines can be used, and a role for oxidative stress in contact allergen-induced IL-18 can be demonstrated.
Acknowledgements. This research was supported by the EU grant SENS-IT-IV #018681.

PS 966 DIFFERENT REGULATION OF INTERLEUKIN-8 EXPRESSION IN HUMAN THP-1 CELLS AFTER EXPOSURE TO ISOEUGENOL *VERSUS* OTHER CONTACT ALLERGENS.

E. Corsini[1], A. Carne[1], V. Galbiati[1], M. Mitjans[2], C. L. Galli[1] and M. Marinovich[1]. *[1]Department of Pharmacological Sciences, Università degli Studi di Milano, Milano, Italy and [2]Dpt Fisiologia, Facultat de Farmacia, Universitat de Barcelona, Barcelona, Spain.*

We have demonstrated in THP-1 cells that all allergens tested selectively induce a rapid p38 MAPK activation and IL-8 mRNA expression. While the proapten isoeugenol fails to induce IL-8 release, all other allergens tested also induced a dose-related release of this cytokine. In the present study we investigated whether this abnormal behaviour of isoeugenol is regulated by the downstream molecules suppressor of cytokine signalling (SOCS) 3, and/or by HuR or tristetraprolin (TTP). THP-1 cells were treated with isoeugenol and, for comparison, with the moderate contact allergen diethylmaleate (DEM), with the strong contact allergen DNCB, and with irritant salicylic acid. Changes in the stability of IL-8 mRMA was analyzed by RT-polymerase chain reaction, while changes in IL-8 mRNA levels were analyzed by quantitative real-time polymerase chain reaction, the release of IL-8 was analyzed by ELISA, and p38 MAPK activation, SOCS-3, HuR and TTP expression by Western blot analysis. Exposure of THP-1 cells to allergens led after 15 min to the activation of p38 MAPK activation and after 1-3 h to IL-8 mRNA expression. However, after 6 h of treatment with isoeugenol the expression of IL-8 mRNA returned to control value, while a sustained expression was still observed in cells treated with DEM and DNCB, suggesting a possible effect on mRNA stability. We could indeed demonstrate a decreased IL-8 mRNA stability in cells treated with isoeugenol. The selective up-regulation of TTP by isoeugenol can explain the reduced IL-8 mRNA expression and lack of protein release.Our data indicate that isoeugenol induced a destabilization of IL-8 mRNA by inducing TTP expression, which resulted in a lack of protein synthesis and release.
Acknowledgements. This research was supported by the EU grant SENS-IT-IV #018681.

PS 967 *IN VITRO* DETECTION OF CONTACT ALLERGENS: DEVELOPMENT OF AN OPTIMIZED PROTOCOL AND PERFORMING OF AN INTERNATIONAL RING STUDY USING HUMAN PERIPHERAL BLOOD MONOCYTE DERIVED DENDRITIC CELLS.

A. G. Schepky, J. Spieker, S. Gerlach, H. Wenck, K. Wittern and H. J. Reuter. *4218-Research Toxicology, Beiersdorf AG, Hamburg, Germany.* Sponsor: R. Fautz.

Allergic contact dermatitis is a delayed T-cell mediated allergic response associated with relevant social and economic impacts. Until now animal experiments (e.g. the local lymph node assay) are supplying most of the data used to assess the sensitization potential of new chemicals. However, the 7th amendment to the EU Cosmetic Directive will introduce a testing ban for cosmetic ingredients after 2013. In vitro alternative methods are thus being actively developed. Although promising results have been obtained with cell lines, their reduced functionality and inherent genomic instability led us to reinvestigate the use of peripheral blood monocyte derived dendritic cells (PBMDCs) for the establishment of a reliable in vitro sensitization test. To solve the issues associated with the use of primary cells, the culture and exposure conditions (cytokine concentrations, incubation time, readout, pooled vs. single donors and cytoxicity) were re-assessed and optimized. Here we propose a stable and reproducible protocol based on PBMDCs. Wider acceptance and feasibility of PBMDCs for the reliable detection of human skin sensitizers were tested in a international ring study. First results will be presented.

PS 968 ASIAN GINSENG (*PANAX GINSENG*) AGGRAVATES ETHANOL TOXICITY IN JAPANESE MEDAKA (*ORYZIAS LATIPES*) EMBRYOGENESIS.

M. H. Haron[1, 3, 2], L. A. Walker[1], I. A. Khan[1] and A. K. Dasmahapatra[1, 2, 3]. *[1]National Center for Natural Product Research, University of Mississippi, University, MS, [2]Environmental Toxicology Research Program, University of Mississippi, University, MS and [3]Department of Pharmacology, University of Mississippi, University, MS.*

Fetal alcohol spectrum disorder (FASD) is an important clinical problem resulting from prenatal exposure to alcohol and one that has serious central nervous system (CNS), cardiovascular, and craniofacial defects affecting the entire lifetime of an individual. Prevention of FASD, other than women abstaining from drinking alcohol during pregnancy, is not known. Significant efforts have been made to educate women about the dangers of alcohol during pregnancy; however, the incidence remained unchanged. The synthetic drugs recommended for the treatment of alcoholism cannot be used by women during pregnancy. The limitations of western medications have led us to investigate on herbal products. In particular, many Chinese herbs including Asian ginseng (Panax ginseng) have therapeutic properties. More recently, black ginseng (produced from red ginseng by nine cycles of steam treatment) has been shown to inhibit ethanol-induced teratogenesis in mouse embryos in vitro. We have developed medaka as an animal model to study FASD and use them for searching herbal medicines that can attenuate toxic effects of ethanol. Fertilized medaka (Oryzias latipes) eggs in standard laboratory conditions (16L: 8D, 25 0C) were exposed to ginseng (PG) root extract (0- 2 mg/ml) for 0-2 day post fertilization (dpf). The calculated IC50 of PG as determined on 10 dpf is 355.3 +/- 1.115 μg/ml (n=3, r2= 0.9001). In separate experiments embryos were exposed to sub lethal concentrations of PG (50 and 200 μg/ml) with or without ethanol (100 and 300 mM) either for 0-2 dpf or 1-3 dpf and examined the mortality 10 dpf. It was observed that PG is able to enhance embryo mortality by disrupting the circulation status of the embryos. It is therefore concluded that PG aggravates some of the ethanol-dependent toxic pathways in medaka leading to decrease in embryo survivability.

PS 969 CYTOTOXICITY OF TOBACCO SMOKE AEROSOLS *IN VITRO*: COMPARISON OF ISO AND HCI SMOKING REGIMES.

K. Scott[1], J. Kilford[2], P. Fowler[2], J. Young[2], I. Crooks[1], D. Dillon[1] and C. Meredith[1]. *[1]Group R&D, British American Tobacco, Southampton, United Kingdom and [2]Covance Laboratories Ltd., Harrogate, North Yorkshire, United Kingdom.*

Recent technological developments in the testing of tobacco products have seen the widespread introduction of equipment that is capable of exposing cultured cells or media to the whole smoke aerosol, adding an additional capability to the well established regimes for testing tobacco smoke total particulate matter *in vitro* for genotoxicity and cytotoxicity. BAT has selected the VC10® (VITROCELL®, Germany) smoking machine, which was designed to directly expose cells to whole smoke. It is widely used in the tobacco industry, requires few cigarettes, delivers smoke to the cells quickly and can be programmed for a range of different smoking methods.
A number of machine smoking methods are available for different regulatory applications. These include the ISO method (35cm^3 puff volume, 2 second duration, 60 second frequency, 0% vent blocking) and the HCI (Health Canada Intense) method (55cm^3 puff volume, 2 second duration, 30 second frequency, 100% vent blocking). In this study, we compared the cytotoxicity *in vitro* of cigarette smoke generated using both ISO and HCI regimes. Reference cigarettes (CORESTA CM6) were smoked in a VC10® smoking machine. BALBc rodent embryo cells were exposed directly to the generated smoke aerosol at the air-liquid interface. Cytotoxicity was evaluated using the Neutral Red uptake assay, following exposure to the smoke aerosol.
Compared to air-exposed controls, a reduction in cell survival of 30% was seen when cells were exposed to the smoke aerosol for 15 minutes, increasing to 40% when the exposure time was increased to 30 minutes. Despite clear decreases in cell

survival using both smoking regimes, there was no clear difference in toxicity between cells exposed to smoke under either ISO or HCI regimes, at exposures of up to 30 minute duration.
Further work is required to define the precise dose of both vapour phase and particulate phase constituents to which the cells are exposed under the stated conditions and to further define time and concentration dependencies.

PS 970 APPLICATION OF SILAC QUANTITATIVE PROTEOMICS FOR PATHWAY DISCOVERY OF IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY COMPOUNDS.

K. L. Leach[1], S. Nadanaciva[1], C. Vazquez-Martin[2], A. Lamond[2] and P. Ajuh[2]. [1]*Compound Safety Prediction, Pfizer, Groton, CT and* [2]*Dundee Cell Products, Dundee, United Kingdom.*

SILAC (Stable Isotope Labeling with Amino Acids in Cell Culture) quantitative proteomics (QP) is a mass spectrometry-based proteomics method that provides accurate and reliable quantitation of changes in protein levels in cells. Drug-induced liver injury (DILI) accounts for 50% of all cases of liver failures in the US, and a significant fraction of those are caused by idiosyncratic DILI (I-DILI) drugs. The cellular mechanisms which contribute to I-DILI are not known. We have applied SILAC QP technology to identify novel cellular proteins and/or pathways that are affected by I-DILI drugs. HepG2 cells were treated with the I-DILI drugs nefazodone (40 uM), glafenine (100 uM) or nimesulide (100 uM) for 72 hours and the cellular proteome was analyzed by SILAC QP. Buspirone (100 uM) and rosiglitazone (100 uM) are not known to cause DILI and were used as control compounds. Over 1800 proteins were identified by at least two peptides per protein in each of the cell lysates. The antidepressant nefazodone caused an increase (2-fold or greater) in the level of 34 proteins and a decrease in the level of 47 proteins whereas buspirone caused much fewer overall changes in protein levels (increases in 14 proteins and decreases in 15 proteins). Nefazodone caused an increase in a number of mitochondrial proteins, including mitochondrial fission protein 1, NADH dehydrogenase, and voltage-dependent anion-selective channel protein, suggesting that changes in mitochondrial function might contribute to the mechanism of toxicity for nefazodone. The cyclooxygenase inhibitors, glafenine and nimesulide, both caused 3-11 fold increases in 4 proteins in the aldo-keto reducatase family. These enzymes are activated by stress caused by electrophiles and reactive oxygen species, and are involved in the bioactivation and detoxification of a number of drugs. Taken together, these results suggest that I-DILI drugs may quantitatively change several proteins and activate specific cellular signaling pathways.

PS 971 EXPERIENCES WITH CYTOTOXICITY ASSAYS TO SELECT STARTING DOSES FOR ACUTE ORAL TOXICITY TESTING.

K. Hempel, A. Schrage, S. Kolle, M. Schulz, B. van Ravenzwaay and R. Landsiedel. *BASF SE, Ludwigshafen, Germany.* Sponsor: A. Doi.

Acute oral toxicity testing is essential for the toxicological assessment of new chemicals. With the objective of reducing and refining animal experiments necessary for determination of acute oral toxicity, we have implemented cytotoxicity data to estimate acute oral starting doses in rats. According to the ICCVAM Validation Study in 2006, we used the neutral red uptake (NRU) method in Balb/c 3T3 fibroblasts after 48 hours exposure to determine the cytotoxicity of about 120 test substances including a broad variety of chemicals and formulations. The predicted starting doses, estimated with the ICCVAM prediction model, were then used in rat acute oral toxicity studies. Comparing the predicted LD50 and the in vivo determined GHS classification, the cytotoxicity assay showed a good prediction only for the low toxic substances (83%, GHS Cat. 4, > 300 – 2000 mg/kg body weight). The overall concordance was rather low (36%), mainly because 76% of the tested substances were classified as low toxic in vitro, but only 34% in vivo. Expanding the prediction +/- one category greatly enhanced the overall concordance to 82% with only 8% overpredicted (in vitro Cat. 3, > 50 – 300 mg/kg, in vivo Cat 5, > 2000 mg/kg) and 10% underpredicted test substances (in vitro Cat. 4, in vivo Cat 1-2, ≤ 50 mg/kg). So far, the use of cytotoxicity data to predict starting doses did not sufficiently contribute to the refinement and reduction in acute oral toxicity testing. As the predictivity of the in vitro test highly depends on specific properties of the tested substances, further analysis considering molecular weight, pH, log POW, solubility, metabolic breakdown and mode of action are necessary and will be presented. Using this data the applicability domain of the cytotoxicity assay for the prediction of acute oral toxicity in rats might be refined.

PS 972 FURAN AND 1, 3-PROPANEDIOL PRODUCE DNA CROSS-LINKS IN TURKEY FOETAL LIVER.

A. M. Jeffrey[1], J. Duan[1], K. D. Brunnemann[1], J. R. Schlatter[2] and G. M. Wiliams[1]. [1]*Department of Pathology, New York Medical College, Valhalla, NY and* [2]*Swiss Federal Office of Public Health, Stauffacherstrasse 101, Zürich, CH-8004, Switzerland.*

Furan is a contaminant formed in food as a result of heating. It is hepatocarcinogenic in rats and mice [1]. The potential genotoxicity of furan in the liver is unclear. Cordelli, *et al* [2] found evidence of liver DNA single strand breaks (SSBs) when B6C3F1 mice were administered a single dose of furan. Genotoxicity can be assessed in an *in ovo* turkey liver assay [3]. In this system, we tested furan and 1,3-propane diol (PD), as a positive control, for their ability to cause DNA-protein cross-links (DPXLs) and DNA fragmentation detectable in the alkaline Single Cell Gel Electrophoresis (COMET) assay. A single injection of furan into turkey eggs at 23 days of development (about 5 days before hatching) produced a reduction in COMET tail length at doses of 2 to 15 μmoles/egg (approximately 75g) in hepatocytes isolated from the foetus 24 hr later. When the same cells were treated with proteinase K to digest the DPXLs, an increase in tail length was observed compared to the corresponding negative controls, indicating the presence of DNA SSBs. PD causes DNA cross-linking in rat liver [4], which the authors considered consistent with conversion of PD to malondialdehyde (MDA). In the eggs, PD showed little toxicity and was used at high doses (up to 300 μmoles/egg), at which it induced both DPXLs and DNA SSBs. This diol, with probable metabolic formation of MDA, showed much less toxicity compared to glutaraldehyde, a known direct acting cross linking agent, which caused significant egg mortality at doses lower than those needed to yield clear evidence of DPXLs and SSBs. In conclusion, furan produced DNA cross-linking and SSBs in foetal turkey liver indicating that these lesions may be involved in its rodent hepatocarcinogenicity. **1**. Natl. Toxicol. Program.Tech. Rep. Ser. ***402***, 1-286 (1993). **2**. Cordelli, E., *et al*, Mutagenesis ***25***, 305-314 (2010). **3**. Perrone, C. E., *et al*, Arch. Toxicol. ***78***, 589-598 (2004). **4**. Summerfield, F. W. and Tappel, A. L. Chem. Biol. Interact. ***50***, 87-96 (1984).

PS 973 RETINOIC ACID DIFFERENTIATED H9C2 RAT CARDIAC CELLS AS A MODEL FOR TOXICITY SCREENING.

H. P. Behrsing, J. Hamre III, M. J. Furniss, D. Mesa and R. E. Parchment. *SAIC-Frederick/NCI-Frederick, Frederick, MD.*

The H9C2 rat embryonic myoblastic cell line has been used to study the effects of toxicants on cardiac muscle, but often in an undifferentiated state. Published articles have indicated that differentiation and method thereof (e.g. inclusion of retinoic acid) has an impact on the expression of cardiac markers (e.g. L-type calcium channel). As part of an effort to establish robust in vitro models for screening of chemotherapeutic compounds, we have characterized the H9C2 cells when undifferentiated and differentiated, in glucose- or galactose-based media. Characterization included staining the cells for muscle-specific markers (cardiac tropinin, myosin light chain 2, and myogenin), and exposure to known cardiac toxicants for IC50 determination using the live cell esterase calcein AM assay, and/or ATP assay. Cells were also treated using etoposide and topotecan and assayed for gamma-H2AX expression, using DAPI and ToPro3 for data normalization. Staining results indicate that differentiation of H9C2 results in the increased expression of cardiac -specific markers, with retinoic acid inclusive conditions yielding a greater increase of expression. Toxicity testing using known mitochondrial toxicants produced expected results, but the differentiated H9C2 acquired a greater sensitivity (e.g. lower IC50 values) than undifferentiated cells for some compounds tested. Gamma-H2AX staining of cells exposed for 6 hr to etoposide (100 μM max, 2-fold dilutions) showed a concentration-dependent increase of gamma-H2AX staining but 6 hr exposure to topotecan (25 μM max, 2-fold dilutions) did not. In conclusion, the H9C2 rat cardiac cell line exhibits favorable characteristics as a cardiac model for toxicity testing when differentiated using retinoic acid. The state of differentiation can impact the cell's expression of organ specific biomarkers and sensitivity to toxicants. Funded by NCI Contract No. HHSN261200800001E.

PS 974 IDENTIFICATION AND CLASSIFICATION OF SKIN SENSITIZER USING PEPTIDE BINDING ASSAY BY LC-MS.

T. Morimoto, T. Higaki, M. Ota, K. Inawaka and S. Kawamura. *Sumitomo Chemical Co., Ltd., Osaka, Japan.* Sponsor: T. Yamada.

We are developing Peptide Binding Assay using LC-MS as an alternative method for the skin sensitization test. In this study, we evaluated the predictive capacities of identification and classification of skin sensitizers in the Peptide Binding Assay. Test chemical and glutathione(GSH:tripeptide) were dissolved in acetonitrile and PBS,

respectively. And these solutions were mixed. In order to identify skin sensitizer, the mixture of test chemical and GSH was incubated for over 12 hours, and then the incubated mixture was analyzed by LC-MS. Where the GSH conjugate with test chemical was detected, the test chemical was judged to be a skin sensitizer. Additionally, in order to classify the potency of skin sensitizer, the mixture of test chemical and GSH was incubated for 2 hours. Then, maleimide was added to the incubated mixture to react with unconjugated GSH. Based on the determination of peak area of GSH conjugate with maleimide by LC-MS, the GSH reactivity of each test chemical was calculated. The GSH reactivity data was compared with GHS classification obtained from animal studies. Compared the results of the Peptide Binding Assay and in vivo study, it is shown that the Peptide Binding Assay gives a high positive predictivity. In addition, it is indicated that most of chemicals which had high GSH reactivity, are classified into Category 1 under the GHS system.Therefore, it is concluded that the Peptide Binding Assay is a useful screening method for evaluation of skin sensitization potency. This assay will be helpful to reduce the number of animals and to shorten the experimental period.

PS 975 A NEW RECONSTITUTED HUMAN CORNEAL EPITHELIUM MODEL FOR THE ALTERNATIVE EYE IRRITATION TEST.

K. Jung[1], S. Lee[2], Y. Ryu[2], H. Jung[2], W. Jang[1], J. Han[3], S. Seok[3], J. Park[3], Y. Son[4], J. Chung[3], Y. Park[1] and K. Lim[1]. *[1], Amore Pacific. Co. R&D Center, Gyeonggi-do, Republic of Korea, [2]Modern Cell&Tissue Technologies Inc., Seoul, Republic of Korea, [3]Seoul National University, Seoul, Republic of Korea and [4]Kyung Hee University, Gyeonggi-do, Republic of Korea.*

Many efforts are being made to develop new alternative in vitro test methods for the eye irritation test. Here we report a new reconstructed human corneal epithelial model (MCTT HCE model) prepared from primary-cultured human limbal epithelial cells as a new alternative in vitro eye irritation test method. In histological and immunohistochemical observation, MCTT HCE model displayed a morphology and biomarker expressions similar to intact human cornea. Moreover, the barrier function was well preserved as measured by high transepithelial electrical resistance, effective time-50 for Triton X-100, and corneal thickness. To employ the model as a new alternative method for eye irritation test, protocol refinement was performed and optimum assay condition was determined including treatment time, treatment volume, post-incubation time and rinsing method. Using the refined protocol, 25 reference chemicals with known eye irritation potentials were tested. With the viability cut-off value at 50%, chemicals were classified to irritant or non-irritant. When compared with GHS classification, the MCTT HCE model showed the accuracy of 88%, sensitivity of 100% and specificity of 77%. These results suggest that the MCTT HCE model might be useful as a new alternative eye irritation test method. #Acknowledgement: This research was supported by a grant (09162KFDA557 and 10182KFDA508) from Korean Food & Drug Administration in 2009-2010.

PS 976 AN ACUTE 1 HOUR EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN (2, 3, 7, 8-TCDD) INHIBITED VITELLOGENIN (ZFVTG 1-3) INDUCTION BY 17α-ETHYNYLESTRADIOL IN ZEBRAFISH (*DANIO RERIO*).

S. M. Bugel[1], L. A. White[2] and K. R. Cooper[2]. *[1]Department of Environmental Sciences, Rutgers University, New Brunswick, NJ and [2]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ.*

Zebrafish (*Danio rerio*) were used to test the hypothesis that exposure to 2,3,7,8-TCDD down-regulates the induction of 3 vitellogenin genes (zfVTG 1-3) by 17α-ethynylestradiol (EE2). Vitellogenin is a hepatic protein required by developing oocytes for growth and maturation and has been demonstrated by our lab to be down-regulated and correlated with decreased egg production in killifish (*Fundulus heteroclitus*) inhabiting Newark Bay (NJ, USA). 2,3,7,8-TCDD was chosen because of its abundance in Newark Bay and for the ubiquity of AhR agonists in aquatic systems reporting reproductive impacts on oviparous organisms. Preliminary experiments established dose-response relationships for significant inductions of zfVTG1 and zfCYP1A mRNA by EE2 and 2,3,7,8-TCDD, respectively ($p \leq 0.05$). To investigate the effect of 2,3,7,8-TCDD on the EE2-dependent induction of zfVTG mRNA expression, zebrafish embryos were either treated with EE2 (1000 pptr) from 6 hpf to 4 days post fertilization or were pre-treated with 2,3,7,8-TCDD (25 and 400 pptr) for 1 hour at 3 hpf and then exposed to EE2. When treated with EE2 alone, zfVTG 1, 2 and 3 were significantly induced 42, 22 and 14-fold, respectively, over control. Exposure to 400 pptr TCDD for 1 hour prior to EE2 treatment significantly inhibited induction of zfVTG 1, 2 and 3, by 97, 98 and 92%, respectively. Exposure to 25 pptr 2,3,7,8-TCDD down-regulated induction of zfVTG 1, 2 and 3 by 85, 75, 58%, respectively. These results demonstrate that acute exposure to 2,3,7,8-TCDD can inhibit the induction of vitellogenin mRNA expression by EE2 and to our knowledge are the first to report a direct effect on vitellogenin gene regulation in an *in vivo* aquatic model. These findings offer insight for reproductive impacts observed in oviparous species inhabiting aquatic systems heavily contaminated by AhR agonists.

PS 977 TOXICOLOGICAL INVESTIGATION OF VOLATILE ORGANIC COMPOUNDS ON NON-MAMALIAN MODEL SPECIES, *DROSOPHILA MELANOGASTER* AND *CAENORHABDITIS ELEGANS* FOR INDOOR AIR POLLUTANTS BIOMONITORING.

Y. Chung[1], H. Eom[1], K. Song[2], S. Kim[3], M. Rhee[4], T. Chon[5] and J. Choi[1]. *[1]University of Seoul, Seoul, Republic of Korea, [2]Korea Conformity Laboratories, Seoul, Republic of Korea, [3]Seoul National University, Seoul, Republic of Korea, [4]Chungnam National University, Seoul, Republic of Korea and [5]Pusan National University, Pusan, Republic of Korea.* Sponsor: D. Ryu.

Indoor air quality has been recognized as a significant health and environment issue. Some air pollutants are reported to occur more frequently and at a higher concentration in indoor air than in outdoor air, including volatile organic compounds (VOCs). In this context, the indoor environment can be of crucial importance because modem society spends most of their time indoors, and exposure to VOCs may result in a spectrum of illnesses ranging from mild to very severe effects. Most of researches on indoor air pollution, however, have been based on the measurement of VOC in the environment and human samples. In this study, we investigated toxicity of VOCs on non-mammalian species. There has been a strong need to reduce, refine or replace mammalian species in toxicological testing with alternative testing methods and non-mammalian models. Fully sequenced genomes of *Drosophila melanogaster* and *Caenorhabditis elegans* have showed an unexpectedly high level of conservation with the vertebrate genome, which makes them attractive animal models for biological studies, including toxicology. In this study, applicability of *D. melanogaster* and *C. elegans* functional genomics tools to indoor air pollutant monitoring was tested; the responses of *D. melanogaster* and *C. elegans* to VOCs exposure were screened across various mutant strains and sensitive mutant strains were selected to be developed as biomonitoring tools for indoor air pollutants. Detailed results will be presented in the conferences.
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PS 978 DETERMINING SKIN SENSITIZATION POTENTIAL OF MEDICAL DEVICE MATERIALS USING A NEW *IN VITRO* METHOD.

D. Keller[1], B. Wallace[1], H. Wagner[1], J. Gorski[2] and J. M. McKim[1]. *[1]CeeTox, Inc., Kalamazoo, MI and [2]North American Scientific Associates, Inc. . (NAMSA), Northwood, OH.*

The ISO 10993 series of standards require that medical devices be evaluated for sensitization potential. This is accomplished using a method that subjects the device to polar and non-polar extraction vehicles and testing the extracts in either the mouse local lymph node assay (LLNA) or the guinea pig maximization test (GPMT). Regulatory issues in the EU, consumer pressure, and testing efficiency make *in vitro* tests desirable. Cells cultured in aqueous media may not provide sufficient exposure for non-polar extracts. The aim of this study was to determine if a new *in vitro* method for detecting chemical sensitization in HaCaT cells could be used in human reconstituted skin models cultured at an air-liquid interface. In order to determine whether the skin models would respond in a manner similar to HaCaT cells, twelve chemicals used in medical devices were selected and evaluated. The chemicals were administered at concentrations ranging from 0.1 to 2500 μM in 0.5% DMSO and incubated for 24 hr at 37°C with 5% CO_2. Direct reactivity was determined by GSH binding. The expression of eleven genes under the control of Nrf2, (e.g. NADPH-dehydrogenase quinone 1 and glutamate-cysteine ligase) was measured by qRT-PCR. The data obtained were analyzed for GSH binding, cell viability, magnitude of gene expression, and potency. These data were processed by a proprietary algorithm and a toxicity index determined. The test compounds, which included toluene diisocyanate and nickel sulfate, were accurately identified

as non-, weak, moderate, or strong sensitizers. These findings were consistent with those reported previously by our laboratory using HaCaT cells. In conclusion, reconstructed human epidermis skin provides a promising model for evaluating polar and non-polar extracts from medical device materials for their skin sensitization potential.

PS 979 NEUROPATHY TARGET ESTERASE (NTE) CODIFYING GEN AS BIOINDICATOR OF EMBRYOTOXICITY IN *IN VITRO* TESTS.

M. A. Sogorb, A. C. Romero, D. Pamies, C. Estevan and E. Vilanova. *Unidad de Toxicología y Seguridad Química, Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, Elche, Alicante, Spain.*

There is a strong request of rapid, safe and reliable alternative methods for detecting teratogenic and embryotoxic chemicals. Neuropathy Target Esterase (NTE) is a protein that seems to be involved in the embryonic development and in the early stages of stem cell differentiation. The exposure of monolayer cultures of D3 mouse embryonic stem cells in differentiation during 5 days to 100 ng/ml of the strong embryotoxic chemical 5-fluorouracil (5-FU) caused a reduction of 50% ($p<0.001$) in the gene expression of NTE, while the cytotoxicity caused by this exposure was only 25%. Non significant cytotoxic exposures to 5-FU (50 ng/ml) also induced a statistically significant ($p<0.01$) reductions in the gene expression of NTE (around 20%). The exposure of D3 cells to 100 ng 5-FU/ml also caused the following statistically significant alterations ($p<0.001$ in all cases): i) a reduction of 96% in the expression of the endoderm marker alpha-fetoprotein; ii) an increase of 13 times in the expression of the mesoderm marker fetal liver kinase 1; iii) a reduction of 50% in the expression of the ectoderm marker nestin; iv) reductions of 70, 66 and 47% in the expression of the vasculogenesis markers vascular endothelial growth factor A, islet-1 and histone deacetylase-7, respectively. It is concluded that the expression of NTE seems to be suitable as bioindicator of embryotoxicity (especially in complementation with other biomarkers of differentiation) and that the use of this bioindicator can be used after only 5 days of exposure, which represents an improvement against the 10 days of exposure needed for the well-established and validated embryonic stem cell (EST) test based on the detection of alterations in the D3 cells towards cardiomyocytes.

PS 980 CO-CULTURE OF HUMAN DOPAMINERGIC NEURONS AND RAT GLIA CELLS AS *IN VITRO* MODEL FOR PARKINSONS DISEASE.

S. Schildknecht, D. Poeltl and M. Leist. *University of Konstanz, Konstanz, Germany.*

The parkinsonian toxin MPTP, respectively its active metabolite MPP+ are widely used models for studies on the selective degeneration of dopaminergic neurons in the substantia nigra. In in vivo models, systemically administered MPTP crosses the blood brain barrier and is converted by astrocytic monoamine oxidase B into MPP+, that ultimately leads to the selective degeneration of dopaminergic neurons. Due to the necessity of repeated administrations of relatively high boli of MPTP, mechanistic investigations on the process of MPTP conversion, its potential pharmacological manipulation, accumulation of MPP+ in the neurons, dopamine release, or the time-dependent degeneration of neurites and cell death are difficult and laborious to investigate in animal models.
We have previously introduced the human dopaminergic cell line LUHMES in combination with MPP+ that reflects the major in vivo features of parkinsonian brains such as energy impairment, oxidative stress, or proteolytic stress.
We herein introduce a new co-culture model composed of the LUHMES cell line and primary rat glia cells as experimental system that closely resembles the characteristics of dopaminergic neurons and allows studies on the interactions between neurons and glia cells under normal and pathophysiological conditions. When MPTP was applied, roughly 50 % was observed to be converted within 6-8 h of incubation. LUHMES cells demonstrated a rapid uptake and significant accumulation of newly formed MPP+. In comparison with MPP+ (5 μM) treated LUHMES mono-cultures, the MPTP (10-20 μM) dependent toxicity of LUHMES in the co-culture model displayed a significant delay in neurite degeneration and cell death. Neurodegeneration by MPTP was prevented by the mixed lineage kinase inhibitor CEP 1347 or by antioxidants such as ascorbic acid.
The LUHMES/glia co-culture model displayed its suitability not only in combination with MPTP, but also with methamphetamine/Fe2+, or as an inflammatory model by lipopolysaccharide or cytokine-mediated activation of glia cells and its impact on LUHMES integrity.

PS 981 POPULATION-BASED QUANTITATIVE HIGH-THROUGHPUT SCREENING (QHTS) FOR CHEMICAL TOXICITY.

S. O'Shea[1], O. Kosyk[1], N. Abdo[1], E. Lock[1], F. Wright[1], R. Huang[2], M. Xia[2], C. Austin[2], R. Tice[3] and I. Rusyn[1]. *[1]Department of Environmental Sciences and Engineering, UNC, Chapel Hill, NC, [2]NIH Chemical Genomics Center, NHGRI/NIH, Bethesda, MD and [3]National Toxicology Program, NIEHS/NIH, Research Triangle Park, NC .*

A shift in toxicity testing from traditional in vivo to higher throughput in vitro methods creates promise to prioritize compounds, uncover mechanisms of toxicity and provide rich data for predictive modeling of adverse health effects in humans. The qHTS paradigm, in which hundreds of compounds may be profiled rapidly in dozens of cell lines and multiple biological targets, is one of the major data streams in computational toxicology. While existing qHTS approaches have been applied to a number of human and rodent cell lines, thus allowing for species and tissue comparisons, the inter-individual genetic variability has not been considered at this scale yet. Our goal was to aid in the development of predictive in vitro genetics-anchored models of chemical-induced toxicity by assessing inter-individual variability and heritability of chemical-induced toxicity phenotypes using cell lines from the Centre d'Etude du Polymorphisme Humain (CEPH) trios assembled by the HapMap Consortium. Lymphoblast cell lines from 27 CEPH trios were exposed to 240 environmental chemicals in 12 concentrations (from 0.26 nM to 46.0 uM). We assessed intracellular ATP production, a measure of cell viability, and caspase-3/7 activity, a marker of apoptosis, 16-40 hrs after treatment with 2-3 replicates. qHTS screening in the genetically-defined population of human cells produced robust and reproducible results, which allowed cross-compound, -assay and -individual comparisons. Some compounds were cytotoxic to all cell types at similar concentrations, whereas others exhibited inter-individual differences in cytotoxicity. The generation of high-quality cytotoxicity data on this large library of compounds using qHTS demonstrates the potential of this methodology to profile a much broader array of assays and compounds while exploring the genetic determinants of inter-individual variability and potentially predicting in vivo biological response.

PS 982 HIGH-THROUGHPUT SCREENING OF TOXCAST™ PHASE I CHEMICALS IN A MOUSE EMBRYONIC STEM CELL ASSAY REVEALS DISRUPTION OF POTENTIAL TOXICITY PATHWAYS.

K. J. Chandler[1], S. Hunter[1], S. Jeffay[1], H. Nichols[1], M. Hoopes[1], M. Barrier[1], N. Sipes[1], N. Kleinstreuer[1], A. Singh[2] and T. Knudsen[1]. *[1]U.S. EPA, Research Triangle Park, NC and [2]Lockheed Martin, Research Triangle Park, NC.*

Little information is available regarding the potential for many commercial chemicals to induce developmental toxicity. The mouse embryonic stem cell (mESC) Adherent Cell Differentiation and Cytoxicity (ACDC) assay is a high-throughput screen used to close this data gap. ToxCast™ Phase I chemicals were evaluated using the ACDC assay in a J1 mESC at 0.0125-12.5uM. Differentiation was measured by In-Cell Western™ of Myosin Heavy Chain (MHC), a cardiomyocyte marker, and cytotoxicity using DRAQ5/Sapphire700. AC50 and AC20 values were computed by curve-fitting methods. Fifty-six of 309 chemicals caused a 50% change in cytotoxicity and/or differentiation in the concentration range tested. Comparison with 548 ToxCast™ assays revealed significant correlations between the inhibition of cardiomyocyte differentiation and perturbation of developmentally regulated genes such as PAX6 ($p\leq0.15$) and BMPR2 ($p\leq0.003$), as well as multiple components of the oxidative stress pathway (ABCG2, HIF1a, NRF2, MTF2, p53) ($p\leq0.15$). A strong association between cell-based ToxCast™ cytotoxicity assays and ES cell cytotoxicity was found. In addition, a multivariate model suggested that ABCG2, a xenobiotic transporter, was a strong predictor of mESC differentiation as were assays that evaluate DNA texture, cell loss, mitotic arrest and steatosis (BA=0.84). Taken together, this work demonstrated the activity of chemicals in ES cells and identified potential disruption of developmental and oxidative stress signaling pathways by a focused group of pesticides. [This work does not necessarily reflect Agency policy.]

PS 983 EVALUATION OF A NOVEL CELL CULTURE EXPOSURE SYSTEM WITH CELL MODELS OF DIFFERENT COMPLEXITY.

F. Glahn[1], A. Rogers[2], S. Kerwien[1], J. Wiese[1], S. Thomas[1] and H. W. Foth[1]. *[1]Institute of Environmental Toxicology, Martin-Luther-University, Halle/Saale, Saxony-Anhalt, Germany and [2]TSE Systems GmbH, Bad Homburg, Germany.*

Even though humans can be exposed to chemicals by various routes of exposure most in-vitro tests are still based on addition of the test substance to the medium. Such conditions do not mimic the in-vivo situation of inhalative exposure to a satisfactory extent. Therefore we evaluated a novel cell culture exposure system (TSE)

permitting treatment of cells on membranes (dry-wet cultures) with aerosols. We used human cell models of different complexity: the lung tumor cell line H322, normal human bronchial epithelial cells (NHBEC) and mini organ cultures of nasal mucosa (MOC). As a model aerosol we applied smoke of 2R4F research cigarettes generated according to ISO3308:2000. Even distribution of the smoke inside the chambers was demonstrated by resazurin-assay. Carbon monoxide was monitored on line in the atmosphere of the exposure units. Cultures were treated with undiluted fresh smoke of 5 cigarettes for 50 min and were kept in an incubator afterwards. CO concentrations exceeded 300 ppm after 12 min and persisted until the end of the exposure period. In experiments with H322 cells viability was measured by MTT-assay and additionally glutathione (GSH)-content was determined by HPLC. All experiments included controls that were exposed to filtered air only. Right after exposure viability was reduced to 93% and it declined to 91, 80 and 57% at 24, 48 and 72h post exposure. GSH-content was increased 1.4 fold after 24h and 1.9 and 2.1 fold at 48 and 72h post exposure. NHBEC were more sensitive in cytotoxicity tests, as exposure to the smoke of 3 cigarettes for 33 min already reduced viability to 93%. We also determined the GSH-content of MOCs exposed to cigarette smoke. In contrast to the results in H322 cells GSH-content in MOCs was reduced to 39 and 25% 24 and 72h after exposure, respectively. These experiments showed the applicability of the evaluated cell culture exposure system to study the effects of aerosols on cell models of different complexity.

PS 984 COMPARATIVE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF AFLATOXIN B1 IN THE WILD TYPE AND MUTANTS OF NEMATODE *CAENORHABDITIS ELEGANS.*

L. Tang[1], S. Xue[2], G. Qian[1], H. Ma[1], P. L. Williams[1] and J. Wang[1]. [1]*Environmental Health Science, University of Georgia, Athens, GA and* [2]*University of Texas at Austin, Austin, TX.*

Aflatoxin B1 (AFB1), a fungal metabolite in food, is one of the most significant mycotoxins. Its potent hepatotoxic and carcinogenic effects involve metabolic activation of parent compound through cytochrome P450 (CYP450) enzymes. The reproductive and developmental toxic effects of AFB1, although were shown in certain animal models, have not been well studied. The nematode *C. elegans* has emerged as an excellent model for toxicological studies due to its short life cycle. Because the complete genome sequence and cell lineage map of *C. elegans* have been identified, genetic manipulation of genes, especially for CYP450 encoding genes, can be made. In this study, toxic effects of AFB1 on the development and reproduction of the wild type and mutant *C. elegans* were investigated. N2 (wild type), VC40 (CYP13A7 gk31 del), VC603 (CYP14A2 gk289 del), and VC249 (CYP14A5 gk152 del), either at 1-day or 3-day old, were treated with AFB1 concentrations at 0-16 mg/L under non-induced and β-naphthoflavone-induced conditions. There are no significant differences on 24 h or 72 h lethality between wild type and mutant *C. elegans* as evidenced by 24 h LC50 of 77.1, 55.9, >100, and >100 mg/L AFB1 and 72 h LC50 of 4.7, 3.9, 5.1, 6.3 mg/L AFB1 in N2, VC40, VC603, and VC249, respectively. AFB1 strongly disturbed the development and reproduction of both wild and mutant *C. elegans*. The worm growth, expressed as development arrested rate (DAR), which equals to (length of control - length of treated)/length of control x 100%, was severely suppressed at AFB1 concentration of 2 μg/mL and above. DAR was 28.4%, 17.1%, 12.4%, and 30.5% for N2, VC40, VC603, and VC249 at 2 μg/mL AFB1, respectively. The reproduction was also significantly affected by AFB1 with 24 h EC50 of 4.1, 1.4, 1.5, and 1.8 μg/mL in N2, VC40, Vc603, and VC249, respectively. Analysis of AFB1 metabolites in medium and worms by HPLC showed different chromatogram patterns between treated and untreated with AFB1 among wild and mutant *C. elegans.*

PS 985 β-CATENIN ACTIVATION IS NECESSARY FOR SURVIVAL OF HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO CIGARETTE SMOKE CONDENSATE.

W. W. Polk. *Research and Development, Lorillard Tobacco Company, Greensboro, NC.* Sponsor: C. Carter.

Bronchial epithelial cells reside in areas of the lung that receive high doses of exposure, undergo significant changes in cell morphology, attachment and motility, and may give rise to non-small cell lung carcinomas (NSCLC) following exposure to cigarette smoke condensate (CSC). A screen of pharmacologic inhibitors in the human bronchial cell line, BEAS-2B, revealed that inhibition of β-catenin decreases the LD100 of CSC from 100μg/mL to 50μg/mL. Since β-catenin is a known proto-oncogene and is a pathological marker strongly correlated with aggressive tumor behavior in several cancers, we hypothesized that β-catenin activation by CSC exposure increases the expression of genes associated with aggressiveness in NSCLC tumors. Using a multiplexed high content screening assay we demonstrated that CSC treatment, in a dose and time dependent manner, increased β-catenin activation. Furthermore, β-catenin activity was selected for, as all remaining cells treated with 50μg/mL CSC or greater had activated β-catenin, whereas control groups and low CSC doses had lower and variable activation of β-catenin. A PCR array of CSC-treated cells demonstrated that the expression levels of genes associated with aggressive NSCLC phenotypes through changes in extracellular matrix remodeling, including the osteopontin, osteonectin and the MMPs are regulated by β-catenin following CSC exposure. This work demonstrates that in Beas-2B cells, CSC-induced β-catenin activation is an early survival response that regulates the expression of multiple genes associated with increasing tumor aggressiveness in models of NSCLC.

PS 986 MOLECULAR EFFECTS OF INORGANIC AND ORGANIC MERCURY IN *CAENORHABDITIS ELEGANS* AND HUMAN CELLS.

M. K. McElwee and J. H. Freedman. *NIEHS, Durham, NC.*

Humans are exposed to both inorganic and organic mercury. While the toxicity of mercury is well established, little is known about how different species act at the molecular level. To address this issue, we employed a toxicogenomics approach using the nematode *C. elegans*. Using sub-, low- and high-toxic exposures to inorganic (HgCl2) and organic (CH3HgCl) mercury, the effects of these mercurials on the steady-state mRNA levels for the entire genome were determined. A total of 473 and 2,865 genes were differentially expressed in the HgCl2 and CH3HgCl (MeHg) treatments, respectively. Hierarchical clustering, principal components, and pattern analyses indicated that the transcriptional responses of the mercurials were unique. The biological function of the up-regulated genes was examined in *C. elegans* using RNAi. The effect of RNAi and mercurial co-exposure on *C. elegans* growth was tested for 599 genes. Knock-down of 18 of these genes affected the growth of mercurial-exposed *C. elegans*. Of these 18 genes, 2 affected growth in response to both mercurials. ABCG2, BACE1, BACE2, CHKA, CHKB, ELOVL3, ELOVL6, GCLC and PARG are human homologs of the *C. elegans* genes that affected growth following mercurial exposure. The effect of sub-, low-, and high-toxic treatments of both mercurials on expression of these genes was examined in SK-N-SH, HepG2 and HEK293 cells. Of the 162 cell-gene-mercury-toxicity combinations tested, 36 resulted in a significant change in gene expression. Of these, 24 were differentially expressed by only one mercurial. The effect on viability of these genes in mercurial exposure was also examined in the cell lines using RNAi. Eleven significant gene-mercury interactions were found. There was not a cell type-gene combination in which exposure to both mercurials affected viability. In whole organism and cell culture studies, HgCl2 and MeHg showed unique effects on transcription and different genes mediated the cellular response. These data indicate that molecular mechanisms of toxicity differ by mercurial.

PS 987 3D CELL CULTURE IMPROVES DETECTION OF OXAZOLIDIONE TOXICITY.

J. Yang[1], X. Xu[1], K. Howe[2], J. Dykens[2] and Z. Cui[1]. [1]*Department of Engineering Science, University of Oxford, Oxford, United Kingdom and* [2]*Drug Safety Research and Development, Pfizer, Sandwich, United Kingdom.* Sponsor: M. Sharpe.

Cell based assays are routinely used to detect potential cytotoxicity of novel compounds during pre-clinical drug development. Conventionally such assays are performed in static monolayer cultures. However, such 2D culture formats lack the 3D structure of the real cellular environment and fail to accurately detect adverse effects of many known human toxicants. To simulate in vivo reality we have been using 3D culture formats with media flow-through capabilities. Using a bioreactor developed at the University of Oxford, we have grown rat adipose-derived stem cells (ADSCs) in a 3D structure under a continuous flow of medium to maintain pH and nutrition. Cytotoxicity of oxazolidiones via inhibition of mitochondrial protein expression is induced by isomers with cis (CONA-04806), but not trans (CONA-04807), stereo chemistry on the heterocycle. Using Alamar blue as a viability index, we evaluated cytotoxicity of the two stereoisomers in 2D vs. 3D culture formats with or without media flow. The isomer with cis stereochemistry was significantly more cytotoxic than the trans isomer in both 3D-perfused or 3D-static culture compared to either 2D-perfused or 2D-static culture. This effect was seen after three days of treatment and continued to six days of treatment. The trans molecule had no such effect with viability being similar in all conditions tested. However, a reduction in cell viability was observed at the highest dose tested (30 μM) in both 2D and 3D culture. The results demonstrate that the 3D perfused system improved the prediction of drug toxicity over 2D culture formats thereby recommending 3D culture for preclinical drug toxicity testing.

PS 988 NNK-INDUCED CYTOKINE ALTERATIONS IN LUNG SLICES FROM LEAN AND DIET-INDUCED OBESE C57BL/6J MICE.

T. Liberati and M. Randle. *Internal Medicine, Southern Illinois University School of Medicine, Springfield, IL.*

Lung cancer is a significant cause of mortality in the U.S., with tobacco use being the primary risk factor. However, only 10% of smokers develop lung cancer suggesting that other risk factors (e.g. genetic profile, metabolic status, and pre-existing lung disease) likely play a role. Studies have indicated that inflammation may contribute to the initiation of lung cancer. However, the inflammatory milieu that promotes carcinogenesis has not been elucidated. Obesity is recognized as a "state of chronic inflammation" due to the presence of abundant mediators in serum and adipose of obese individuals. To investigate how obesity may alter the inflammatory profile of lung and its innate immune response to a carcinogen, we evaluated the impact of NNK, a tobacco carcinogen, on lung slices prepared from C57BL/6J mice that were maintained on either standard or high-fat diets. Following instillation with culture medium containing 1% LMP agarose, 750µM slices were placed on Netwell™ inserts with supplemented DMEM at 37° C and 10% CO_2. After 24 h, slices were treated with 0, 10, 50 or 250 µM NNK. Slices were harvested 4 and 24 h later to measure LDH release into the medium and concentrations of cytokines/chemokines in homogenates and medium. Compared with lean mice, slices from mice with diet-induced obesity (DIO) showed greater LDH release, regardless of NNK dosage. In slices not exposed to NNK, cytokine concentrations in homogenates and media were higher in DIO mice as compared with lean mice. This was most apparent after 48 h *in vitro*. Homogenates and media from the lung of DIO mice had higher concentrations of G-CSF, IL-6, CXCL14 and CXCL1 and lower concentrations of CXCL10 than did samples from lean mice. After exposure to NNK for 24 h, homogenates and media from DIO mice had higher concentrations of IL-6, CXCL9, CXCL14 and CXCL1 than did those from lean mice. We conclude that lung from DIO mice shows an "inflammatory" profile when maintained *in vitro* as lung slices and responds to a known carcinogen in an augmented manner as compared with lung from lean mice.

PS 989 IRRITATION IN THE LLNA: 2000 MICE EAR THICKNESS MEASUREMENTS DEPENDENCE ON VEHICLE AND HCA TREATMENT.

J. L. Moore, M. R. Carathers, D. R. Cerven and G. L. DeGeorge. *MB Research Laboratories, Spinnerstown, PA.*

The Local Lymph Node assay (LLNA) is known to be susceptible to false-positives caused by irritating test substances. We have incorporated ear thickness measurements on Days 1, 3 and 6 of the LLNA in order to measure dermal irritation-induced ear swelling. Known LLNA false-positive irritants SLS and BAC caused increases in ear swelling on Day 6 (when compared to Day 1) of 25% or greater (>1.25-fold). In addition, the default positive control Hexylcinnamaldehyde (HCA) has irritation potential, which was confirmed at concentrations above 10% HCA. Interestingly, all common LLNA vehicles (Acetone:Olive Oil 4:1(AOO), Acetone, Ethanol, Dimethylacetamide:Acetone:Ethanol 4:3:3 (DAE433), etc.) did not affect ear swelling through Day 6, with the notable exception of Dimethylsulfoxide (DMSO). DMSO-treated mouse ear swelling was increased over 15% during the six-day experimental term. This implicates DMSO as moderately irritating and possibly a problematic vehicle for the LLNA.

PS 990 THE CYTOTOXIC AND INFLAMMATORY RESPONSE OF BRONCHIAL EPITHELIAL CELLS EXPOSED TO CIGARETTE WHOLE SMOKE AND VAPOUR AT THE AIR-LIQUID INTERFACE.

G. J. Phillips, L. E. Haswell, G. Foss-Smith, K. Hewitt, S. Corke, D. Azzopardi, M. D. Gaca and C. Meredith. *Group R&D, British American Tobacco, Southampton, United Kingdom.*

As part of our approach to harm reduction we are developing potential reduced-exposure products (PREPs). Currently only outline guidelines for the evaluation of PREPs exist and as such, a case by case approach to PREP assessment is required. The early stage in this process includes the biological evaluation of cigarette whole smoke and vapour, rather than just the particulate content, using *in vitro* models that measure some physiological or disease associated endpoint. To achieve this, a whole smoke exposure system has been developed that allows for the exposure of cells at the air-liquid interface to be undertaken. In this study, bronchial epithelial cells (NCI-H292) were initially grown to confluence on semi permeable membranes (Transwell™). The cells were then raised to the air-liquid interface by removal of the covering culture medium and exposed for 30 minutes to cigarette whole smoke (Reference Cigarette 3R4F) over a range of predetermined dilutions (1:5 to 1:400). An equivalent set of cells were exposed to the vapour phase component of whole smoke by removal of the particulate fraction using an inline Cambridge filter pad. Following a 24 hour recovery period under culture medium, cytotoxicity and secreted mediators of inflammation (IL-6, IL-8 and MMP1) were measured using the neutral red assay, ELISA and the Meso Scale Discovery (MSD™) platform. Results demonstrate that vapour constitutes 82% of the total cytotoxicity derived from whole smoke exposure. At subtoxic dilutions of whole smoke (>1:250), the concentrations of inflammatory mediators were increased compared to cells exposed to equivalently diluted vapour. These results show that vapour is a significant cytotoxic and inflammatory component of cigarette whole smoke. Further studies are underway to assess the effects of PREPs on this model.

PS 991 THE USE OF *IN VITRO* TOXICITY DATA AND PHYSIOLOGICALLY BASED KINETIC MODELING TO PREDICT DOSE-RESPONSE CURVES FOR *IN VIVO* DEVELOPMENTAL TOXICITY OF GLYCOL ETHERS IN RAT AND MAN.

J. Louisse[1, 2, 3], E. de Jong[4, 5], J. J. van de Sandt[2], B. J. Blaauboer[5], R. A. Woutersen[1, 2, 3], A. H. Piersma[4, 5], I. M. Rietjens[1, 3] and M. Verwei[2, 3]. [1]*Division of Toxicology, Wageningen University, Wageningen, Netherlands,* [2]*TNO Quality of Life, Zeist, Netherlands,* [3]*WUR/TNO Centre for Innovative Toxicology, Wageningen, Netherlands,* [4]*National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands and* [5]*Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands.*

The regulatory assessment of systemic toxicity is almost solely performed using animal models, providing in vivo dose-response curves. If in vitro toxicity data are to be used for human risk assessment, in vitro concentration-response curves should be translated to in vivo dose-response curves that can be used to set a point of departure for deriving safe exposure limits. The present study shows an approach to extrapolate in vitro concentration-response curves to in vivo dose-response curves for developmental toxicity by combining in vitro toxicity data and in silico kinetic modeling. A physiologically based kinetic (PBK) model was developed, describing the kinetics of four glycol ethers and their embryotoxic alkoxyacetic acid metabolites in rat and man. In vitro toxicity data of these metabolites derived in the embryonic stem cell test (EST) were used as input in the PBK model to extrapolate in vitro concentration-response curves to predicted in vivo dose-response curves for developmental toxicity of the parent glycol ethers in rat and man. The predicted dose-response curves for rat were found to be in concordance with the embryotoxic dose levels measured in reported in vivo rat studies. These predicted dose-response curves for rat might be used to set a point of departure for deriving safe exposure limits in human risk assessment. Combining the in vitro EST toxicity data with a human PBK model allows the prediction of dose-response curves for human developmental toxicity. This approach could therefore provide a means to reduce the need for animal testing in human risk assessment practices.

PS 992 EVALUATION OF MEDAKA AS AN ANIMAL MODEL TO STUDY AUTISM SPECTRUM DISORDER.

M. Wu[2, 1], I. A. Khan[1] and A. K. Dasmahapatra[1, 2]. [1]*National Center for Natural Product Research, University of Mississippi, University, MS and* [2]*Department of Pharmacology, University of Mississippi, University, MS.*

Autism spectrum disorder (ASD) is an umbrella term used to define a broad spectrum of atypical social, cognitive and verbal behaviors along with repetitive and sometimes self-injurious actions. The behavioral criteria used to diagnose ASD are not sufficient to justify the diagnosis. Moreover, there is no appropriate medicine available for the treatment of ASD. The psychoactive drug risperidone is prescribed for ASD patients, however, majority of them are used alternative medicines for the prevention of this disorder. We predict that two major barriers exist in understanding the pathogenesis of ASD: (1) the absence of a defined phenotypic marker or gene product specific to ASD, and (2) the lack of an appropriate animal model that can display core symptoms of ASD. We are evaluating medaka as an animal model to identify exogenous phenotypic features of ASD using valproic acid (VPA) as the inducer. Further we will evaluate the potency of natural products as an alternative to prevent ASD. Fertilized medaka eggs at specific phase of central nervous system development (1-3 day post fertilization, dpf) were exposed to different concentrations of VPA for 48 hours and the mortality was assessed on 10 dpf. The calculated IC50 as observed on 10 dpf is 10.69 +/- 1.87 mM. The hatchling were used for craniofacial cartilage staining and observed that several neurocranial cartilages are smaller in size compared to controls. We predict that studies in craniofacial cartilage development and differentiation may be able to identify a unique phenotypic feature that can be used to diagnose ASD exogenously

PS 993 CHLORPYRIFOS DISRUPTS THE BLOOD-BRAIN BARRIER BY ALTERING TIGHT JUNCTION AND TRANSIENT RECEPTOR POTENTIAL CHANNEL GENE EXPRESSIONS.

W. Li and M. Ehrich. *Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.*

The blood-brain barrier (BBB) is formed by specialized endothelial cells, a layer lining microvessels in the central nervous system. Chlorpyrifos (CPF) is a widely used organophosphate insecticide. We have previously shown that exposure to low concentrations of CPF decreased electrical resistance across the BBB in vitro, indicating loss of BBB integrity. In the present study, we examined the possibility that CPF disrupted BBB at the gene expression level. Rat brain endothelial cells (RBE4) were co-cultured with rat astrocytes on membrane inserts to form an in vitro BBB. RBE4 cells were then exposed to CPF (0.1-10 μM) for 2, 4, and 12 hours. Total RNA was extracted from RBE4 cells using a RNA purification kit. Quantitative real-time PCR for gene expression of tight junction proteins claudin5, scaffold proteins zona occludins (ZO1), and a transient receptor potential channel (TRPC4) for calcium were examined. Gene expressions for all three were decreased 2 hours after exposure to CPF, with TRPC4 decreased by 43%. ZO1 expression was decreased by 13%. In contrast to the decrease, still seen with TRPC4 and claudin5 at 4 hours (34% and 45%, respectively), ZO1 gene expression was increased by 12%. At 12 hours after CPF exposure, TRPC4 and claudin5 expression were back to baseline levels, but ZO1 was increased by 15%. The results indicate that altering gene expression for claudin5, TRPC4, and ZO1 by CPF may directly contribute to BBB disruption.

PS 994 *CAENORHABDITIS ELEGANS* AS AN ALTERNATIVE MODEL TO EVALUATE NANOMATERIAL TOXICITY.

L. Komatsu[1], N. Olejnik[1], K. M. Tyner[2] and R. L. Sprando[1]. *[1]U.S. FDA, Laurel, MD and [2]CDER, U.S. FDA, Silver Spring, MD.* Sponsor: T. Flynn.

There is a growing concern regarding the toxicity of nanoparticles. Currently, the majority of nanotoxicity research is focused on cell culture systems. In vivo systems are extremely complicated, and it is both time consuming and expensive to evaluates the toxicity of nanomaterials with different physical and chemical properties in animals. Therefore, there is a need for alternative nanotoxicity test model. C. elegans has been widely utilized in toxicity testing. A review of the literature has suggested that toxicological effects observed in worms may, in many cases, reflect effects observed in mammalian models. The objective of the present study was to develop C.elegans as an alternative model to assess nanomaterial toxicity using high throughput screening assay (C.elegans Complex Object Parametric Analyzer and Sorter, COPAS) and liquid culture. 10 nm silver nanoparticles were selected as a model test compound. In the current study, COPAS parameters Time of Flight and Extinction were utilized to monitor growth and development in C. elegans exposed to various concentrations of nano-silver (10 nm) for 72 h. Light reflection by aggregated nano-silver particles inside the worm allowed us to evaluate the localization of nanosilver in treated C. elegans by confocal microscope. Nano-silver induced considerable toxicity in C. elegans. A dose-dependent decrease in growth and development was observed in nano-silver treated worms, and confocal microscopic images indicated that nanosilver particles were distributed throughout the worm body. Approximately 50 % of worms treated with nano-silver at 50 mg/L exhibited protruding vulva phenotype. In addition, there was a decrease in the numbers of eggs laid by worms treated with nano-silver. Our preliminary data suggest that axenic liquid cultured C. elegans in combination with confocal microscopy and high-throughput screening using the COPAS could be a powerful tool in assessment of nanoparticle toxicity.

PS 995 EFFECTS OF DIETARY PHENOLICS AND BOTANICAL EXTRACTS ON HEPATOTOXICITY-RELATED ENDPOINTS IN HUMAN AND RAT HEPATOMA CELLS AND STATISTICAL MODELS FOR PREDICTION OF HEPATOTOXICITY.

Y. Liu[2], T. Flynn[1], M. Ferguson[3], E. Hoagland[1] and L. Yu[2]. *[1]Division of Toxicology, U.S. FDA, Laurel, MD, [2]Nutrition & Food Science, University of Maryland, College Park, MD and [3]Division of Public Health & Biostatistics, U.S. FDA, College Park, MD.*

Several dietary supplements have been implicated in clinical liver toxicity. Toxicity assessment of herbal dietary supplements is difficult because they are typically complex mixtures of phytochemicals. In the present study, seventeen pure plant polyphenols were tested at a concentration of 50 μg/mL in both human HepG2 and rat MH1C1 hepatoma cells using a battery of eight toxicity endpoints. Cluster analysis was used to group the polyphenols into four clusters for each cell type. Comparison of overall and individual liver activity of tested polyphenols and botanical extracts on both human and rat hepatoma cell lines showed significant species differences for some endpoints. However, the cluster membership was similar across both cell types with the majority of polyphenols clustering with the untreated control group (cluster 1). Each cell type produced a cluster of compounds with reported liver toxicity (cluster 2). For the HepG2 cells these compounds were gallic acid, tannic acid and nordihydroguaiaretic acid (NDGA). For the MH1C1 cells these compounds were tannic acid, NDGA, epicatechin and epigallocatechin gallate. Five herbal extracts were prepared and then tested at a concentration of 1000 μg/mL. Using the cluster model developed with the polyphenols, in the HepG2 cells green tea was assigned to cluster 2 and the remaining four extracts to cluster 1. In the MH1C1 cells, green tea and thyme were assigned to cluster 2, cinnamon to cluster 4 (rosmarinic acid only), and juniper berry and peppermint to cluster 1. The data suggest that this in vitro model may be useful for identifying hepatotoxic polyphenols and for screening herbal preparations for potential liver toxicity. [Supported by grant #U032 to L.Y. and T.F. from the Joint Institute for Food Safety and Nutrition at the University of Maryland]

PS 996 EXPANDED PREVALIDATION FOR THE ISOLATED RABBIT EYE (IRE) ASSAY.

F. Guerriero[1], C. Seaman[2], A. Whittingham[3], A. Else[3], E. Adriaens[4], R. Guest[3] and M. Olson[1]. *[1]H, S&P, GlaxoSmithKline, Research Triangle Park, NC, ^{2}H, S&P, GlaxoSmithKline, Ware, United Kingdom, [3]Harlan Laboratories, Shardlow, United Kingdom and [4]Adriaens Consulting, Aalter, Belgium.*

Two organotypic assays for detecting severe ocular irritant effects of chemicals, BCOP and ICE, have achieved full validation status and entry into the OECD test methods framework. A similar method, the IRE, is also used as a screen for detecting ocular irritants, but requires an expanded dataset to more positively suggest readiness for formal validation. GSK studies of 45 substances selected from proprietary pharmaceutical processes and for which in vivo eye irritation data were available showed sensitivity of 83% (77% for solids, 100% for liquids) and specificity of 71% overall (92% solids, 38% liquids) in the IRE. Further work is underway to address areas for which lack of data has hampered validation. A two-phase study is being conducted with a training set of 21 substances followed by evaluation of a further 23 substances, all drawn from ECETOC and ICCVAM databases. All substances in the training set have been assessed and outcomes have stimulated development of a refined model for classifying the multi-endpoint IRE results as predictive of 3 GHS ocular irritation categories (Cat. 1, 2, and not classified). Using this model for concordance assessment with the training set showed sensitivity of 75% (67% for solids, 100% for liquids) and specificity of 100% overall. Application of the refined model for retrospective assessment of all available GSK data showed 81% sensitivity (75% solids, 100% liquids) and 75% specificity (93% for solids, 50% for liquids). Furthermore, evaluation of training set data suggested 1) excellent reproducibility between independent replicate experiments, 2) differential duration of exposure may aid in optimizing results (e.g., 5 sec. exposure duration for liquids, 10 sec. for solids), 3) the applicability domain, although broad, may exclude substances acting via macromolecular binding and solids acting in part by physical irritation, 4) histology corroborates and may refine conclusions reached by observation of corneal swelling, opacity, and fluorescein staining.

PS 997 CYTOTOXICITY AND GENOTOXICITY OF PHENAZINE IN HUMAN CELL LINES.

C. F. McGuigan[1], S. Gabos[2] and X. Li[1]. *[1]Division of Analytical & Environmental Toxicology, University of Alberta, Edmonton, AB, Canada and [2]Alberta Health and Wellness, Edmonton, AB, Canada.* Sponsor: R. Bull.

Phenazine, a bacterial metabolite, was recently identified as a drinking water disinfection byproduct (DBP). Little is known about its effects on mammalian cells; therefore, this study is to examine cytotoxicity and genotoxicity in the human carcinoma cell lines HepG2 (liver) and T24 (bladder). We investigated the cytotoxic effects of phenazine (1.9μM-0.12mM) using impedance-based real-time cell electronic sensing. This provides a dye- and label-free measurement of cell viability, proliferation, or morphology in real-time, called "cell index" (CI). Increasing CI indicates cell proliferation, growth, and/or attachment; decreasing CI may indicate cell death, detachment, and/or a cytostatic state. We observed a concentration-dependent decrease in CI in HepG2 cells exposed to phenazine, but no effect on T24 cells at these concentrations. Significant decreases in CI compared to the control

group (one-way ANOVA, $p<0.05$) were observed in HepG2 cells treated with ≥31μM phenazine, but not in T24 cells, at 24 hours exposure. Proliferation analysis with 5-bromo-2-deoxyuridine (BrdU) showed that phenazine significantly inhibited proliferation compared to the solvent control group (one-way ANOVA, $p<0.05$) of HepG2 cells after 24 hours exposure at all tested concentrations, but no difference was observed in T24 cells until phenazine ≥31μM. Genotoxicity of phenazine was tested based on tail moment values from alkaline single-cell gel electrophoresis after 24 hours exposure. T24 cells showed a significant (one-way ANOVA, $p<0.05$) increase in tail moment compared to the control group at ≥62μM phenazine, indicative of DNA damage. No significant DNA damage was detected in HepG2 cells. These results suggest that phenazine could affect different cells via different mechanisms: the cytotoxic effect observed in HepG2 cells appears independent of DNA damage, while T24 cells experience sub-cytotoxic DNA damage.

PS 998 *IN VITRO* EXPOSURE OF ORGANOTYPICAL 3D EPITHELIAL TISSUES TO CIGARETTE SMOKE AS A POTENTIAL ALTERNATIVE TO RODENT INHALATION STUDIES.

D. Weisensee, B. Kurkowsky, M. Hebestreit and S. Wagner. *PHILIP MORRIS Research and Development, PHILIP MORRIS Research Laboratories GmbH, Cologne, Germany.* Sponsor: E. Roemer.

Airway epithelium is the initial barrier affected by toxic gases or particles in the atmosphere, such as cigarette smoke (CS), which induces, among other things, inflammatory processes that can lead to COPD and tissue remodeling. Here we describe an *in vitro* test system which examines the toxicological effects of repeated CS exposure on organotypical 3D epithelial tissues derived from differentiated human airway epithelial cells. Tissues were exposed at the air liquid interface to either fresh air or to mainstream smoke from the Reference Cigarette 3R4F in the Vitrocell® system for 1-3 cycles of 20 min each to a CS concentration of 20% (12-well format) or for 7, 14, 21, and 28 min to a CS concentration of 10%, 15%, or 20% (24-well format). Cell viability, as determined by resazurin reduction 24h after CS exposure, was not adversely affected. For morphological characterization of the reconstituted tissue H&E, AB-PAS, and immunohistochemical P63 (basal cells) stainings were performed. These stainings showed intact basal and pseudostratified epithelium with mucociliary phenotype similar to *in vivo* proximal airway epithelium. Gene expression of hemoxygenase-1 (HO-1), cytochrome P450 1A1 (CYP1A1), heat shock 70kDa protein A6 (HSPA6), and transforming growth factor β1 (TGF β1) was strongly up-regulated (qRT-PCR) under all conditions. Matrix metalloproteinase-1 (MMP-1), an enzyme possibly involved in the pathogenesis of COPD, was dose-dependently increased (ELISA measurement). Results for HO-1 and CYP1A1 and MMP-1 are comparable to *in vivo* data from previous rodent inhalation studies which showed increased HO-1 and CYP1A gene expression in the lung and increased MMP-1 secretion in the lavage fluid of rats exposed to CS for up to 1 month. The *in vitro* study design presented here serves as a potential alternative to rodent inhalation studies for the investigation of the toxicology of CS and supports the 3R strategy of refinement, reduction, and replacement of animal experimentation.

PS 999 EVALUATION OF MALE RAT ENDOCRINE TOXICITY USING THE *IN VITRO* RAT TESTICULAR CELL LINE R2C.

R. Kuk[1], P. S. Giffen[2], S. B. Laffan[1] and D. J. Stanislaus[1]. [1]*Reproductive Toxicology, Safety Assessment, GlaxoSmithKline, King of Prussia, PA and* [2]*SA Pathology, Preclinical Development, GlaxoSmithKline, Ware, Hertfordshire, United Kingdom.*

Certain chemicals are known modulators to male steroidogenic pathways that can be utilized to stage-manage an in vitro system as a model system to elucidate where in the pathway a test article is disrupting. Rat testicular cell line R2C has been characterized to produce all hormones along the steroid pathway from cholesterol transport to production of testosterone (T), and was utilized as a system to evaluate drug toxicity and investigate the mechanism of action. Compound X (an acetylcholine receptor modulator) was given to Sprague Dawley rats and decreased plasma testosterone levels by up to 50% of concurrent control after repeat dosing for up to 1 month without any corresponding change in prostate, seminal vesicle or epididymal weights. To investigate the mechanisms of action, R2C cell line was utilized. A range of concentrations of compound X up to 50 μM were evaluated. All concentrations were tested for cytotoxicity before hormone analysis. Compound X did not have any biologically significant effect on metabolic viability at concentrations < 50 μM. Positive controls: ketoconazole (30 μM) and aminoglutethimide (100 μM), along with other reagents: anastrozole (1 μM) and 22R hydroxycholesterol (12.5 μM) did not have any treatment related effect on metabolic viability in the R2C cells. Compound X produced decreases in testosterone, androstenedione,17- hydroxyprogesterone (OHP4), progesterone, and pregnenolone production in rat R2C cells. The series of experiments indicated that compound X (up to 50 μM) did not inhibit enzymatic steps (P450 side chain cleavage (scc), 3β-HSD, CYP17 and P450 aromatase) between cholesterol processing and synthesis of T. Thus, results suggested that compound X had an inhibitory effect in a step (or steps) that occur before cholesterol cleavage.

PS 1000 THE DEVELOPMENT OF AN *IN VITRO* ASSAY TO PREDICT SKIN SENSITIZATION BASED ON ROS PRODUCTION.

K. Saito, M. Miyazawa, Y. Nukada, H. Sakaguchi and N. Nishiyama. *Kao Corporation, Tochigi, Japan.* Sponsor: J. Avalos.

Recently, Reactive Oxygen Species (ROS) have been shown to induce dendritic cell migration and contact hypersensitivity (Na et al., 2007). We investigated the use of ROS as a useful marker for assessing skin sensitization in a murine dendritic cell line. In this study, we developed a new in vitro assay to predict skin sensitization based on ROS production in human monocytic leukemia cell line THP-1 cells. THP-1 cells were pre-loaded with a ROS sensitive fluorescent dye, CM-H2DCFDA, for 15 min at 37°C. After staining, the medium containing dye was removed and cells were incubated with the test compound under a serum-free condition for 30 min and then fluorescence was measured by flow cytometry. First phase, we treated the THP-1 cells with either a sensitizer, 2,4-dinitrochlorobenzene (DNCB), or a non-sensitizer, benzalkonium chloride (BAC). DNCB but not BAC induced >6-fold ROS production. Next, we evaluated 18 sensitizers (e.g. oxazolone, benzoyl peroxide, cinnamic aldehyde) and 12 non-sensitizers (e.g. sodium lauryl sulfate, lactic acid, glycerol) for their potential to increase ROS production. 15 out of 18 sensitizers induced >2-fold ROS production with a dosage causing >90 % viability, but only 2 out of 12 non-sensitizers did. Three sensitizers (phthalic anhydride, propyl gallate and hexyl cinnamic aldehyde) did not induce >2-fold ROS production. Propyl gallate is known as a ROS scavenger while and hexyl cinnamic aldehyde is a water-insoluble substance. Consequently, setting the 2-fold ROS production as tentative positive criteria, sensitivity, specificity and accuracy were determined to be 83.3%. Also, 5 compounds (citral, abietic acid, cyclamen aldehyde, ethyleneglycol dimethacrylate, butyl glycidyl ether) out of 6 sensitizers categorized as "weak" in local lymph node assay were judged as "positive" in this assay. These results indicated that THP-1 cells-based ROS assay was a sensitive and mechanism-based tool to predict skin sensitization.

PS 1001 A TIERED APPROACH COMBINING FOUR *IN VITRO* ASSAYS FOR PREDICTING EYE IRRITATION POTENTIAL OF CHEMICALS.

K. Hayashi[1], T. Abo[1], K. Ooshima[2], T. Hayashi[2], T. Komano[2], R. Fautz[3], T. Simion[4] and H. Sakaguchi[1]. [1]*Kao Corporation, Tochigi, Japan,* [2]*Kanebo Cosmetics Inc., Kanagawa, Japan,* [3]*Kao Professional Salon Services GmbH, Darmstadt, Germany and* [4]*Kao Brands Company, Cincinnati, OH.*

For the assessment of ocular irritation, one in vitro alternative test may not completely replace the Draize test. In the present study, we examined the predictive potential of a tiered approach analyzing the results from four in vitro assays (i.e., the STE test with SIRC cells, the EpiOcular assay with 3D models, the HET-CAM assay with chorioallantoic membrane (CAM) of hen's eggs and the BCOP assay with isolated bovine corneas) for assessing GHS eye irritation rankings. Around 50 chemicals including alcohols, surfactants, and esters were selected with a balanced GHS ranking and a wide range of chemical classes. The irritation category of non-irritant (NI) or irritant (I) for the chemicals were evaluated in the STE test (Takahashi et al. 2008), the EpiOcular assay (Harbell et al. 2009) and the HET-CAM (a) assay (Hagino et al. 1999). If the chemicals were soluble in the STE test vehicle, the results of the STE test were reviewed. However, if not soluble, then the results from the EpiOcular or the HET-CAM (a) were reviewed. If the chemical was classified as a "NI" in any of these assays, it was considered to have a GHS rankings of "not classified". If the chemical was classified as an "I", the classification was subsequently confirmed by reviewing the results from the BCOP (Gautheron et al. 1992) and HET-CAM (b) (Kalweit et al. 1987) as described in the background review document (ICCVAM-NICEATM 2006). If the classification was "severe", the chemical was considered a GHS "Cat. 1". For those chemicals whose

classifications were "non-severe", these were considered to be GHS "Cat. 2". The tiered (bottom-up) approach combining the STE test, the EpiOcular assay / the HET-CAM (a) assay and the BCOP assay allowed the evaluation of GHS eye irritation rankings with a favorable predictivity (accuracy > 75%, under prediction rate < 5%). From these results, this tiered approach might be a promising alternative method for predicting eye irritation.

PS 1002 TOWARDS ANIMAL-FREE TESTING FOR SKIN SENSITIZATION: IN-HOUSE VALIDATION OF FOUR METHODS: MUSST, H-CLAT, KERATINOSENS, AND DPRA.

C. Bauch[1, 2], T. Eltze[1], E. Fabian[1], S. N. Kolle[1], C. Pachel[1, 3], T. R. Hernandez[1], B. Wiench[1, 4], C. J. Wruck[2], R. Landsiedel[1] and B. van Ravenzwaay[1]. *[1]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany, [2]Department of Anatomy and Cell Biology, RWTH Aachen University, Aachen, Germany, [3]Technical University Kaiserslautern, Kaiserslautern, Germany and [4]Faculty of Chemistry, Pharmaceutics, and Geosciences, Johannes Gutenberg University Mainz, Mainz, Germany.* Sponsor: A. Van Cott.

Allergic contact dermatitis is induced by repeated skin contact with an allergen. Assessment of the skin sensitizing potential of chemicals, agrochemicals, and cosmetic ingredients is crucial to define their safe handling and use. Up to now, animal tests have been used to identify skin sensitizing potential. Animal welfare as well as the 7th Amendment to the Cosmetics Directive and REACH demands animal free alternatives. The mechanisms of induction and elicitation of skin sensitization are complex. To account for the multitude of events in the induction of skin sensitization an in vitro test system will consist of a battery of various tests. Currently, we perform in house validations of four in vitro assays addressing three different events during induction of skin sensitization. 1) The peptide reactivity assay (DPRA, 1) using synthetic peptides and HPLC analysis. 2) Two dendritic cell based assays on the cell lines U 937 (MUSST) and THP-1 (h-CLAT) and flow cytometric detection of the maturation markers CD54 and/or CD86 (2, 3). 3) ARE-dependent gene activity in the reporter gene cell line KeratinoSens. We present the results of these assays with more than 40 substances of known sensitizing potential including the performance standards defined for the LLNA. The sensitivity, specificity and accuracy of individual tests were obtained by comparison to human epidemiological data as well as to data from the local lymph node assay.

PS 1003 ASSESSMENT OF AIRWAY GENOTOXICITY POTENTIAL USING THE EPIAIRWAY *IN VITRO* HUMAN AIRWAY MODEL AND THE COMET ASSAY.

A. Armento, J. DeLuca, Y. Kaluzhny, H. Kandarova, M. Klausner and P. J. Hayden. *MatTek Corp., Ashland, MA.*

Determination of genotoxicity potential is an important consideration for safety assessment of chemicals that may be inhaled during exposure to consumer products, occupational chemicals or environmental pollutants. Commonly used in vitro genotoxocity assays produce a high rate of false positive results, limiting their practical utility for predicting human genotoxicity hazard. For assessment of organ-specific genotoxicity, 3D in vitro human tissue models which have in vivo-like barrier function and metabolic capability are expected to have improved biological relevance and predictive ability. Due to recently enacted legislation including REACH and a ban on animal testing of cosmetics by the 7th Amendment to the Cosmetics Directive, predictive organ specific genotoxicity tests are urgently needed. Previous work has focused on development of skin specific genotoxicity tests including the micronucleus assay and the comet assay. In the current poster, we describe development of an airway specific genotoxocity assay using the EpiAirway model and the Comet Assay. The EpiAirway model is produced from normal human airway epithelial cells and reproduces the psudeostratified mucociliary phenotype of in vivo proximal airways. The model has functional tight junctions, in vivo-like barrier properties and in vivo-like xenobiotic metabolizing capabilities. EpiAirway tissues were digested with (0.25%) trypsin to produce cell suspensions for use in Comet Assay experiments. 100 comets were visually scored per tissue and each condition was run in duplicate tissues. Visually scoring was conducted by assigning values for % tail DNA on a 5 category scale (0 being undamaged DNA to 4 >80% DNA in tail). Untreated control samples produced low background comet scores. Treatment of the EpiAirway tissues with prototypical genotoxins such as methyl methane sulfonate (MMS) prior to digestion produced statistically significant, dose-dependent increases in % tail DNA. Thus, the EpiAirway Comet Assay appears to be a promising approach to in vitro airway genotoxicity testing.

PS 1004 APPLICATION OF PRECISION-CUT LIVER SLICES FOR THE INVESTIGATION OF XENOBIOTIC-INDUCED CELL PROLIFERATION.

E. Fabian[1], F. Schuck[2, 1], C. Jaeckh[1], S. Groetters[1], B. van Ravenzwaay[1] and R. Landsiedel[1]. *[1]Expermental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany and [2]Johannes Gutenberg-University, Mainz, Germany.*

Precision-cut liver slices (PCLiS) are a commonly used in vitro system for the assessment of metabolism and transport of xenobiotica in the liver. Within the current study, PCLiS were evaluated for their applicability to detect xenobiotic induced cell proliferation, since cell proliferation is a non-genotoxic contributor to hepatocarcinogenesis. Therefore, a rotating culture system was established and characterized by viability parameters such as membrane integrity (LDH, ALT), energy status (ATP) and mitochondrial activity (MTT). In this system FCS-supplemented Williams' Medium E sustained liver slices for four to six days. Intracellular LDH activities of slices showed activities of more than 200 U/L/mg wet weight after three days of culture and declined to 5% of this activity after six days. ATP assessment showed similar results. Slices contained levels of about 100 pmol ATP/mg wet weight after three days of cultivation and declined to 3% of this level after six days. Data from the MTT assay confirmed these findings. The mitochondrial reduction declined to levels of about 40% of the control after six days of cultivation. Based on the established culture conditions, the system was tested for its capability to induce hepatocyte proliferation after treatment with Wy-14,643, a known PPARα agonist in rodents. The proliferation in cultured liver slices was assessed as an integral over five days by BrdU co-incubation and immuno-staining of histological sections of PCLiS by counting stained cells. Incubations of 1 to 100 μM Wy-14,643 resulted in a concentration-dependent increase of proliferating cells up to a factor of 1.5-fold compared to the control. This study demonstrated a proof of principle for the detection of xenobiotic-induced induction of cell proliferation in PCLiS.

PS 1005 EVALUATION OF XENOBOTIC METABOLISM IN HUMAN RECONSTRUCTED SKIN MODELS FOR GENOTOXICITY TESTING.

C. Jaeckh[1], V. Blatz[1], E. Fabian[1], K. Reisinger[2], B. van Ravenzwaay[1], S. Trappe[3] and R. Landsiedel[1]. *[1]BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany, [2]Henkel AG and Co., KGaA, Duesseldorf, Germany and [3]Bundesinstitut for Risikobewertung, Berlin, Germany.*

Skin models are used to assess the irritation potential of chemicals. Chemicals and their metabolites are also reported to induce DNA-damage. Information on chemical biotransformation within the skin is therefore demanded for genotoxicity testing. Metabolic capacities of native rat, human and, porcine skin have been reviewed by Oesch et al. 2007. Less is known about the metabolic capacity of human reconstructed skin models, a potential alternative in vitro test system to replace rodent in vivo testing.
The objective of this study was to investigate enzymatic activities of three reconstructed skin models compared to native human skin with the perspective to select a regulatory accepted human skin model for genotoxicity testing. Enzyme activities were investigated by incubation of model substrates within S9 or microsomal fractions of the epidermal skin model EpiDerm™(MatTek), the full-thickness skin model EpiDermFT™(MatTek) and the Phenion® full-thickness models Phenion®FT (Henkel). Selected enzymes included CYP-enzymes, Flavin- dependent monooxygenases, UDP-glucuronyltransferase as well as N-acetyltransferases. Contradictive to reported CYP-enzyme transcription, CYP-enzyme activities remained under the limit of quantification. FMO, UDP-GT and NAT–catalyzed reactions were quantified in all reconstructed skin models, however with differing catalytic activity. In summary, EpiDerm™, EpiDermFT™ and Phenion®FT show certain metabolic capacities. Catalytic activities differ between the skin models and in between skin layers. The results obtained recommend the use of full-thickness rather than epidermis skin models. Finally, we demonstrate that biotransforming capacity of reconstructed skin models allows the detection of genotoxic potential of promutagenic substances using the COMET Assay. We acknowledge funding from the German Federal Institute of Research and Education (BMBF; 0315226D).

PS 1006 CUTANEOUS METABOLISM.

J. Eilstein, G. Léreaux, J. Meunier, J. Leclaire and D. Duché. *Life Sciences Research-Safety Research Department, L'Oréal Advance Research, Aulnay-sous-Bois, France.* Sponsor: H. Toutain.

According to the literature, the metabolism of other organs than the liver such the skin seems much less studied. Indeed, skin represents the major protective barrier of the body to the environment and chemicals exposure but is not really yet consid-

ered as an organ involved in xenobiotic metabolism. It appears to be a tissue of weak catalytic activity generating less diverse metabolites and less funny reaction mechanisms. However, this assertion could be due to the lack of specific tools to study skin metabolism such as particular sample preparation protocols or analytical methods which are accurate and sensitive enough. Thus, as skin is the largest organ of the human body, even if weak enzymatic activities are observed, they can become consequent when considering its total surface area. Consequently, research on skin metabolism would require a real scientific effort and dynamism to characterize skin metabolizing enzymes and their activities. In addition, the 7th European amendment to the cosmetic directive forbids the use of animal testing to assess the effectiveness and safety of new cosmetics. This policy has forced the cosmetic industry to develop in vitro tools as alternative methods to animal experiments. Reconstructed human skin models are a part of them. For these reasons, these models have to be characterized and compared with normal human skin in terms of metabolic capabilities. This work presents a review of the L'Oreal research strategy and main results in the characterization of skin metabolic equipment and its catalytic capabilities. Thus, characterization for the expression of several enzymes (CYP450, Esterases, NAT, GST, UGT, SULT…) and their catalytic activities (Apparent Km, Vmax and Vmax/Km) in various reconstructed skins were compared to normal human skin samples.

PS 1007 ASSESSMENT OF COMBINATIONS OF ANTIANDROGENIC COMPOUNDS VINCLOZOLIN AND FLUTAMIDE IN A YEAST BASED REPORTER ASSAY.

S. Kolle[1], G. Krenrich[2], B. van Ravenzwaay[1] and R. Landsiedel[1]. *[1]BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany and [2]BASF SE, GVC/S Scientific Computing, Ludwigshafen, Germany.* Sponsor: A. Doi.

Humans are exposed to a complex combination of various chemicals, cosmetic ingredients, drugs, biocides, pesticides and natural substances. The combined toxicological effects of two or more substances (e.g. endocrine active compounds) can be greater (synergistic) or smaller (antagonistic) than that predicted on the basis of dose-addition or response-addition. Endocrine disruptors may interact with hormone receptors thereby modifying the physiological function of endogenous hormones. The need to assess chemical mixtures for combined effects of compounds with endocrine activity is currently discussed for regulatory risk assessment.
We have used an in house validated yeast based androgen receptor transactivation assay (YAS) to assess the combinatorial androgen receptor mediated antiandrogenicity of vinclozolin, and flutamide. In the presence of the androgen dihydrotestosterone (5x10-9 mol/L) we have assessed mixtures of vinclozolin and flutamide in the concentration ranges of 10-9 to 10-4 mol/L. We have performed seven independent experiments in which each mixture was tested in quadruplicate. Absorbance measurements obtained were normalized to reporter activity of dihydrotestosterone alone. Both vinclozolin and flutamide were antiandrogens of similar potency in the YAS assay. In the concentration range tested the two antiandrogens vinclozolin and flutamide did not interact.

PS 1008 SCREENING FOR ENDOCRINE DISRUPTORS IN PRODUCT DEVELOPMENT: TIERED TESTING USING *IN VITRO* AND *IN VIVO* ASSAYS.

B. van Ravenzwaay, S. Kolle, R. Buesen, H. Kamp and R. Landsiedel. *BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany.* Sponsor: A. Doi.

The development and application of in vitro screening tools significantly enhanced our ability to detect indicators of significant toxicological potential. The early detection of toxicity during substance development is a key element in the decision making process to promote or discontinue development of new chemicals / active ingredients and thereby optimizes the efficient use of resources and reduces animal testing. Here, we present our approach to reduce and refine animal testing to detect endocrine disruptors. Most endocrine disruptors interact with hormone receptors or steroid biosynthesis/ metabolism, thereby modifying the physiological function of endogenous hormones. We have implemented the use of yeast based transcriptional activation assays as well as a mammalian cell based steroidogenesis assay. The yeast estrogen (YES) and yeast androgen screening (YAS) assays were in house validated for receptor mediated agonistic and antagonistic activities and are used to screen substances during early development. Furthermore, we utilize the H295R cell line to detect compounds exerting endocrine effects by changing the level of hormone production as a screening assay based on OCSPP 890.1550. Following a series of in vitro studies, those substances for which development is continued are subject to a first in vivo testing (normally a 7 or 14 day oral study). We combine this first in vivo study with a metabolome analysis of the blood. This analysis allows us to confirm or disconfirm amongst many other modes of action, the potential in vivo endocrine effect of a compound. Therefore, specific, but additional in vivo studies such as the Hershberger assay (OECD TG 441) or uterotrophic assay (OECD TG 440) are rarely needed. This tiered approach combined with the use of modern toxicological techniques has significantly increased the efficient allocation of resources and reduced animal use and thereby presents an important contribution to animal welfare in product development.

PS 1009 THE KERATINOSENS ASSAY TO DETECT SKIN SENSITIZERS BASED ON NRF2-DEPENDENT GENE ACTIVITY: EXPLORING THE INTER-LABORATORY REPRODUCIBILITY AND THE APPLICABILITY DOMAIN.

A. Natsch[1], C. Bauch[2], G. Ellis[1], L. Foertsch[4], F. Gerberick[4], K. Norman[3], R. Landsiedel[2], S. Onken[5], H. Reuter[5], A. Schepky[5] and R. Emter[1]. *[1]Givaudan Schweiz AG, Duebendorf, Switzerland, [2]BASF SE, Ludwigshafen, Germany, [3]The Institute for in vitro Sciences, Inc. .(IIVS), Gaithersburg, MD, [4]Procter & Gamble Inc., Cincinnati, OH and [5]Beiersdorf AG, Hamburg, Germany.*

The Nrf2-Keap1 pathway is a key toxicity pathway induced by skin sensitizers. The KeratinoSens assay is based on a reporter cell line with a luciferase gene under the control of Nrf2. Luciferase induction and cytotoxicity are measured to test for skin sensitization potential. To investigate inter-laboratory reproducibility, the assay was transferred to four additional labs which measured full dose-response curves for 28 chemicals. A very good within- and between-laboratory reproducibility was found: For 24 chemicals all labs reported congruent results, for 2 chemicals 4 of 5 labs obtained congruent results and the dose response curves were highly reproducible. The assay has previously been validated on a list of 67 chemicals of known sensitization potential with an accuracy of 85%. The applicability domain was now further explored by testing specific chemical classes with the following conclusions: (i) Skin sensitizing air-oxidized terpenes are discriminated from their non-allergenic parent molecules based on differential gene induction, (ii) typical photosensitizers absorbing in the UVA-range tested positive after activation by UVA, and (iii) irritating surfactants do not induce luciferase activity (or do so only at clearly cytotoxic concentrations) and can thus be discriminated from sensitizers. This assay reflects the 'Toxicity Testing in the 21th Century' paradigm since it comprises (i) high-throughput format, (ii) full-dose response measurements and (iii) is targeting a relevant toxicity pathway. Recently, a number of toxicological endpoints have been found to be related to the induction of the Nrf2-pathway. Therefore the KeratinoSens assay as an Nrf2-reporter assay which has gone through thorough inter-laboratory trials may even find application beyond the screening for potential skin sensitizers.

PS 1010 THE MYELOID U937 SKIN SENSITIZATION TEST (MUSST) FOR THE PREDICTION OF SKIN SENSITIZATION POTENTIAL.

J. Ovigne, C. Piroird, D. Verda, F. Rousset, S. Martinozzi-Teissier and J. Meunier. *L'Oréal Research, Aulnay-sous-Bois, France.* Sponsor: H. Toutain.

Background: Skin sensitization is a delayed type allergy consisting of a cellular immune reaction to small molecular weight chemicals. So far, animal test methods such as the local lymph node assay (LLNA) were used to predict the skin sensitization potential of unknown chemicals. In line with the 3Rs concept, in vitro alternatives methods are being developed based on the early events of the skin sensitization process. One of these is the capacity of skin dendritic cells to recognize a chemical as a danger. Since the late nineties, L'Oréal has actively worked on the Myeloid U937 Skin Sensitization Test (MUSST) which models this early event by measuring the up-regulation of CD86 expression, a co-stimulatory receptor. Methods: U937 cells are incubated in a 96-well plate with a concentration range of a chemical for 48h. The CD86 expression as well as cell viability (propidium iodide exclusion) are measured by flow cytometry. The MUSST prediction model is defined as: threshold of viability = 70% viable cells, threshold of positivity = 150% of vehicle control, a chemical is classified as sensitizer if it induces a dose-dependent up-regulation of CD86 expression in two concordant experiments. Results: 1) The MUSST prediction is exemplified with 2 sensitizers (Phenyl benzoate, Methyl chloro isothiazolinone), 2 pre/prohaptens (ethylene diamine, eugenol) and 2 non sensitizers (lactic acid, benzaldehyde) correctly classified by the assay. 2) The predictive performances of the MUSST are evaluated with a panel of 50 reference chemicals (31 sensitizers and 19 non sensitizers) against the LLNA data. With the 40 classified chemicals (10 are inconclusive), the MUSST displays a concordance of 83% with 81% sensitivity and 84% specificity. Conclusion: The MUSST is an efficient assay for the skin sensitization hazard characterization, promising as a tool to be integrated within a battery of assays to perform a skin sensitization risk assessment. These data supported the submission of the MUSST to and its acceptation by ECVAM for a pre-validation.

PS 1011 *IN VITRO* GENE EXPRESSION PROFILING TO PREDICT SKIN SENSITIZING POTENTIAL.

J. van der Veen[1, 2], J. Ezendam[1], T. Pronk[1, 2], R. Vandebriel[1], J. Pennings[1] and H. van Loveren[1, 2]. [1]*Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, Netherlands and* [2]*Department of Health Risk Analysis and Toxicology, Maastricht University, Maastricht, Netherlands.*

The upcoming ban on animal testing in the Cosmetics Directive and costliness of the high number of animals required under REACH, have prompted a high demand for the development of alternative methods to replace the currently used animal models for skin sensitization. One promising *in vitro* system is the use of gene expression profiling in human keratinocytes (KC), which are key players in the initiation of skin sensitization. In a previous study using the human keratinocyte cell line HaCaT the *in vitro* effects of sensitizers on gene expression profiles were evaluated. That study showed that a set of 13 genes was able to classify sensitizers with 70% accuracy. Pathway analysis showed that pathways related to oxidative stress were significantly upregulated by sensitizers. To improve classification and to further explore the relevance of this pathway the experiment was extended with additional chemicals. HaCaT cells were exposed to 18 sensitizers and 8 irritants in a concentration leading to 80% viability after 24 hours exposure. Gene profiling was used to classify sensitizers and find regulated pathways. Classification using the Random Forest algorithm in a leave-one-compound-out cross validation scheme resulted in a gene list that was able to classify skin sensitizers with 96% accuracy. The only incorrectly classified compound was the irritant sodium lauryl sulphate, which is a false-positive compound in the murine LLNA. Interestingly, it proved possible to correctly classify the false-positive compound xylene and the false-negative compound nickel. Pathway analysis was performed using ToxProfiler. Pathways related to the antioxidant response and the innate immune response were identified. In conclusion, KC gene profiling can be applied to identify contact sensitizers and may thus be a useful *in vitro* model to predict skin sensitization potential of chemicals.

PS 1012 COMPARATIVE INVESTIGATION OF PHOTOTOXICITY OF CHEMICAL COMPOUNDS BY USING THE STANDARD BALB 3T3 NRU PHOTOTOXICITY TEST AND A THREE-DIMENSIONAL HUMAN SKIN MODEL (EST 1000).

A. Heppenheimer and A. H. Poth. *Genetic and Alternative Toxicology, Harlan Cytotest Cell Research GmbH, Rossdorf, Germany.*

Where substances are intended for use in personal care products applied to the skin an assessment of potential phototoxic hazard is required. The initial test is the measurement of a UV/visible absorption spectrum to identify absorption at relevant wavelength, followed by in an in vitro assay for phototoxicity, the Balb 3T3 neutral red uptake phototoxicity test (OECD 432). However, this test has its limitations, as non-hydrosoluble chemicals can be tested only at low concentrations due to their lack of aqueous solubility and also many complex mixtures or formulations cannot be tested. Consequently, it does not take into account the bioavailability of test chemicals topically applied to skin and in many cases e.g. after having a phototoxic effect in this test system, such information is required. To overcome these limitations, the use of reconstructed skin models is an interesting alternative and a useful follow-up test. In the present study the EST 1000 skin model (Cell Systems, Germany) was used. Chloropromazine and sodium dodecyl sulphate were used as reference compounds. 6-methylcoumarin (6-MC) and Rose Bengal were selected as model compounds. 6-MC is known to possess a photoxic potential in vivo but it does not show effects in vitro in the Balb 3T3 test, whereas Rose Bengal is known to show no in vivo photoxicity, but effects in the Balb 3T3 test. Both compounds were comparatively evaluated in the the Balb 3T3 neutral red uptake phototoxicity test and the EST 1000 skin model. In contrast to the Balb 3T3 model, the human skin equivalents were able to predict the phototoxic potential of both compounds. The obtained data indicate that the EST 1000 model is a useful model for the prediction of phototoxicity and also useful as a follow-up test.

PS 1013 FUNCTIONAL *IN VITRO* ASSAYS OF DIFFERENT CELL SOURCES ARE ABLE TO PREDICT SPECIFIC IMMUNOSUPPRESSIVE PROPERTIES OF CHEMICAL COMPOUNDS.

A. Fischer, L. Koeper and H. Vohr. *Toxicology, Bayer Schering Pharmacology Wuppertal, Germany.* Sponsor: H. Ellinger-Ziegelbauer.

An increasing aim in safety assessment of chemicals and drugs is to reduce, refine and replace animal testing. Great efforts were and still are made to evaluate potential adverse effects of compounds on the immune system *in vitro*. Regarding immunosuppression, most methods are based on mitogen stimulation assays, although it has been demonstrated that a functional assay is one of the most sensitive indicators for primary immunotoxic effects *in vivo*.
The Mishell-Dutton culture (MD), a well known method to investigate immunosuppression, represents a functional assay that requires the interaction of different cell types to generate an immune response *in vitro*. To our knowledge the test has never been considered as an *in vitro* alternative to the existing animal tests.
In a previous study we were able to demonstrate the ability of MD assays with mouse spleen cells to correctly predict different immunosuppressant compounds[1].
To expand its potential, we modified the Mishell-Dutton culture and are now able to measure the *in vitro* antibody response of peripheral blood mononuclear cells (PBMC) as well as spleen cells of different species.
With this *in vitro* assay we are able to demonstrate immunosuppressive effects of test items and to clearly discriminate between specific immunosuppression and non-specific cytotoxicity. Preliminary data show an excellent concordance between species as well as different cell sources.
[1] L.-M. Koeper and H.-W. Vohr, 2009. Functional assays are mandatory for a correct prediction of immunotoxic properties of compounds *in vitro*. Food and Chemical Toxicology 47, 110-118

PS 1014 PRECISION-CUT LIVER SLICES AS AN *EX VIVO* MODEL TO STUDY IDIOSYNCRATIC HEPATOTOXICITY IN MOUSE AND HUMAN.

M. Hadi[1], Y. Chen[1], M. Stitzinger[2], H. Emmen[2] and G. Groothuis[1]. [1]*Pharmacokinetics, Toxicology & Targeting, University of Groningen, Groningen, Netherlands and* [2]*Toxicology, NOTOX BV, s'-Hertogenbosch, Netherlands.* Sponsor: A. Vickers.

Idiosyncratic drug reactions (IDRs) are adverse drug reactions that are rare, sporadic, unpredicted by clinical trials, unrelated to drugs' pharmacology and occur without relation to time or dose. IDRs may arise from drug interaction with inflammation that render the liver more sensitive to injury resulting in increased toxicity. With the aim to develop a translational model to unravel the mechanism behind IDRs and to find biomarkers that can detect them, we used mouse (M) and human (H) precision-cut liver slices (PCLS) to study the influence of inflammatory reactions on the toxicity of drugs. PCLS technology is receiving increased attention as a potential *ex vivo* toxicological model because PCLS retain the normal tissue architecture of an intact liver with all its cell types in their natural environment. PCLS from M and H were incubated with paracetamol (APAP), diclofenac (DF), ketoconazole (KT) or clozapine (CZ) alone and in the presence of lipopolysaccharide (LPS), an inflammation inducer. Cell viability [ATP] and cytokine production [CBA] were assessed. APAP, DF, and CZ (but not KT) were more toxic in M than H. LPS had no influence on APAP and DF toxicity in M or H. APAP and DF slightly increased LPS-induced TNF-α production in M but strongly reduced it in H PCLS and both decreased the LPS-induced IL-1β production in both species. In contrast, toxicity of KT (only in M) and CZ (in both M and H) was aggravated in the presence of a non-toxic dose of LPS and an increase in LPS-induced IL-1β production was observed here. In conclusion, clear species differences were identified in the effect of the drugs on toxicity and on inflammatory reactions due to LPS. Toxicity of KT (only in M) and CZ (in both M and H) was aggravated by LPS, not observed in the cases of APAP or DF. A correlation appeared between increased toxicity and increased LPS-induced IL-1β production in the co-incubation of LPS and drug. Based on these results, PCLS appear to be a promising *ex vivo* model to study mechanisms behind IDRs.

PS 1015 TRANSPORT OF CHLORPROMAZINE ACROSS CACO-2 CELLS AND MEASURING THE FREE CONCENTRATION BY ND-SPME.

J. Broeders, B. Blaauboer and J. Hermens. *Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands.*

Animal experiments are used during drug development to determine the pharmacokinetic parameters of a compound. The use of in vitro and in silico techniques could replace some of these in vivo tests. However, to improve the predictability of these in vitro tests, it is also necessary to fully understand the kinetic behavior of the compound in the in vitro system. Therefore, the aim of this project, part of the European project Predict-IV, is to improve our understanding of the kinetics of compounds in in vitro test systems.
The kinetic behavior of chlorpromazine (CPZ) in the Caco-2 cell system was studied. Bi-directional transport was measured and the influence of bovine serum albumin (BSA) added basolaterally was determined. A negligible depletion-solid phase microextraction (nd-SPME) method was developed to measure the free concentration of CPZ in the medium. This concentration was used to correct the Caco-2 permeability results. All samples were analyzed by HPLV-UV.

Transport of CPZ across the Caco-2 cell monolayer was concentration-dependent. The average efflux ratio was 3.7, confirming that CPZ is a substrate for active efflux transporters. The addition of BSA decreased the secretory apparent permeability (Papp), lowering the efflux ratio to below 1. The nd-SPME measurements showed that 94% of chlorpromazine was bound to proteins (Kbsa=276L/kg). Correction of the secretory transport values for protein binding of CPZ increased the Papp, giving an efflux ratio above 1 again. Acceptable recovery values (>75%) could only be obtained when the amount of CPZ in all 3 compartments of the in vitro system (medium, cells and well plastic) were taken into account.
In conclusion, BSA needs to be added to the basolateral compartment of the Caco-2 cell system, to better mimic the in vivo situation. Then, when transport of lipophilic compounds is measured, the Papp should be corrected for protein binding. Additionally, mass balance calculations of lipophilic compounds should include the amount inside the cells and bound to the well plastic.

PS 1016 INTERNATIONAL VALIDATION STUDY OF AN *IN VITRO* CELL PROLIFERATION TEST METHOD FOR SCREENING POTENTIAL ESTROGENIC AGONISTS AND ANTAGONISTS IN MCF-7 CELLS.

F. Deal[2], W. Casey[1], P. Ceger[2], D. Allen[2], C. Yang[3], M. Nakamura[4], H. Kojima[5], A. Ono[5], H. Yoon[6], S. Han[7] and W. Stokes[1]. *[1]NICEATM, NIEHS, Research Triangle Park, NC, [2]ILS, Inc., Research Triangle Park, NC, [3]CertiChem Inc., Austin, TX, [4]Hiyoshi Corp., Omihachiman, Japan, [5]JaCVAM, Tokyo, Japan, [6]NIFDS, KFDA, Seoul, Democratic People's Republic of Korea and [7]KoCVAM, Seoul, Democratic People's Republic of Korea.*

The MCF-7 Cell Proliferation test method developed by CertiChem Inc. (CCi) uses a human cell line that endogenously expresses estrogen receptor (hER-alpha and hER-beta) to screen for substances that may induce or inhibit cell proliferation via ER-mediated pathways. NICEATM, in collaboration with JaCVAM and KoCVAM, coordinated an international validation study to evaluate test method accuracy and reliability. Three laboratories (one each in the U.S., Japan, and Korea) tested ICCVAM recommended reference substances with well-characterized in vitro ER data. An initial study using a subset of these substances was conducted at CCi to refine protocols and demonstrate intralaboratory reproducibility and accuracy of the test method. Multi-phased interlaboratory studies were subsequently initiated at laboratories in Japan and Korea to demonstrate transferability and further evaluate test method accuracy and reliability. Phases 1 and 2 of the interlaboratory study were used to demonstrate proficiency, establish historical databases, and to evaluate intra- and interlaboratory reproducibility by testing 12 substances in three independent experiments at both laboratories. Phase 3 testing of 14 additional ICCVAM reference substances (tested once at each laboratory) provided data for a more comprehensive evaluation of interlaboratory reproducibility and accuracy. Phase 4 testing, conducted only at CCi, completed testing for the entire list of 78 ICCVAM reference substances. Results from this validation study will be used as the basis for ICCVAM recommendations on usefulness and limitations of in vitro Endocrine Disruptor test methods, and to develop performance standards for the expedited validation of functionally and mechanistically similar test methods. Supported by NIEHS Contract N01-ES-35504 and SBIR44ES014806.

PS 1017 FINAL RESULTS OF AN INTERNATIONAL VALIDATION STUDY OF AN *IN VITRO* ER TA TEST METHOD IN BG-1 CELLS.

W. Casey[1], P. Ceger[2], D. Allen[2], G. Clark[3], P. Pazos[4], E. Grignard[4], J. de Lange[4], S. Bremer[4], M. Nakamura[5], H. Kojima[6] and W. Stokes[1]. *[1]NICEATM, NIEHS, Research Triangle Park, NC, [2]ILS, Inc., Research Triangle Park, NC, [3]XDS, Inc., Durham, NC, [4]ECVAM, Ispra, Italy, [5]Hiyoshi Corp., Omihachiman, Japan and [6]JaCVAM, Tokyo, Japan.*

The LUMI-CELL® ER (BG1Luc4E2) stably transfected estrogen receptor (ER) transcriptional activation (TA) assay uses the human ovarian cancer cell line, BG-1, that expresses both human hER-alpha and hER-beta to screen for substances that may induce or inhibit estrogenic activity in vitro. NICEATM, in collaboration with ECVAM and JaCVAM, coordinated an international validation study to evaluate the accuracy and reliability of the test method. Three laboratories (one each in the U.S., Europe and Japan) tested ICCVAM recommended reference substances with well-characterized in vitro ER TA data. Subsets of this list were used to evaluate test method accuracy and reliability. Phases 1 and 2 were used to demonstrate proficiency, establish historical databases in each laboratory, evaluate intra- and interlaboratory reproducibility, and identify protocol refinements prior to initiating Phases 3 and 4, where the remaining reference substances were tested. Overall accuracy for identifying in vitro ER agonists was 86% (36/42), with false positive and false negative rates of 20% (2/10) and 13% (4/32), respectively. For in vitro ER antagonists, overall accuracy was 89% (16/18), with false positive and false negative rates of 7% (1/15) and 33% (1/3), respectively. These results will be used to provide the basis for draft ICCVAM recommendations on the usefulness and limitations of the BG1Luc4E2 test method for review by an expert peer panel in March 2011, as well as to develop performance standards for the expedited validation of functionally and mechanistically similar test methods. Results from this study will also be used to support the development of an OECD performance based test guideline for ER TA test methods. Supported by NIEHS Contract N01-ES-35504.

PS 1018 AFLATOXIN B_1 INDUCED DNA DAMAGE IN TURKEY AND CHICKEN EGG FOETAL LIVER CELLS.

J. G. Williams[1], U. Deschl[2] and G. M. Williams[1]. *[1]Department of Pathology, New York Medical College, Valhalla, NY and [2]Boehringer Ingelheim Pharmacology KG, Boehringer Ingelheim Pharmacology KG, Biberach/Riss, Germany.*

Avians are highly sensitive to certain toxins. In fact, aflatoxins, such as aflatoxin B_1 (AFB), a metabolite of the mold *Aspergillus flavus*, were discovered as the cause of Turkey X disease which affects several avian species. AFB is DNA-reactive and hepatocarcinogenic in rats. We previously used an *in ovo* turkey liver assay for assessment of chemical genotoxicity [1]. Here we compare DNA damage by AFB in chicken and turkey foetal livers using the alkaline single cell gel (comet) assay [2]. Single injections into eggs of two different doses (0.062 and 6.2 μg) of AFB were examined under both short term (4 hour) and long-term (4 day) exposure to foeti from chickens at 18 days and turkeys at 24 days of development, approximately 3 and 4 days before hatching, respectively. Liver cells were prepared from the foeti by mincing into fine pieces and allowing settling to leave a suspension of cells that were used for the comet assay. In the alkaline comet assay, AFB produced dose-related DNA damage in the foetal liver cells of both turkeys and chickens at 4 hours, which were reduced by 4 days, indicating repair. Turkey foetal liver cells appeared to be slightly more susceptible to AFB damage, although no differences in the survival between chicken and turkey foeti were observed. The *in ovo* avian liver genotoxicity assay is a facile *in vivo* model for assessing genotoxicity either by nucleotide postlabeling [1] or the comet assay. 1. Perrone CE, *et al.* (2004) Arch Toxicol. ***78***:589-98. 2. Tice RR, *et al.* (2000) Environ Mol Mutagen ***35***:206-21.

PS 1019 TRACE METALS ALTER HISTONE METHYLATION PATHWAYS IN MOUSE EMBRYONIC STEM CELLS.

S. R. Gadhia and F. A. Barile. *Pharmaceutical Sciences, St. John's University College of Pharmacy, Queens, NY.*

The fetal basis of adult disease (FeBAD) states that fetal exposure to (co)carcinogens initiate processes that result in abnormal cell growth in adult life. Environmental exposure to metals fulfills this characterization by stimulating mutations or epigenetic changes in fetal life, resulting in DNA damage or histone modifications later in development, respectively. These perturbations alter gene expression, change transcription rates or interfere with DNA repair mechanisms. We tested the hypothesis that metals alter epigenetic pathways in the course of cancer induction, by measuring cytotoxicity and histone lysine mono-methylation in mouse embryonic stem (mES) cells. Cell viability, total histone protein, and H3K27 mono-methylation were quantified in differentiated cultures of mES cells after exposure to arsenic, copper, cadmium, nickel, mercury, lead) and lithium, for 1-hr and 24-hr, followed by a recovery period. The data demonstrates that maximum cell death occurred during the first few hours of exposure at about 24-hr IC50 concentrations. Overall, 24-hr exposure of cells to As, Cd, Hg and Ni decreased cell proliferation to a greater extent than total histone protein. Whereas Ni and Cd exposures for 24-hr significantly increased total histone protein production per cell, As and Hg exposures did not, suggesting that the latter specifically target total histone protein production. In addition, low dose acute exposure to As, Cd, Hg and Ni decreased mono-methylation of lysine (K27) residue on histone H3 when compared to control cells. Thus, if mono-methyl H3K27 (H3K27me1) is associated with transcriptional activation, and metals decrease mono-methylation of H3K27, then these metals are capable of suppressing transcriptional activation. This supports the contention that trace quantities of metals are capable of suppressing pathways that maintain transcriptional activation, thus altering chromatin structure. The pathway contributes to understanding the basis of cancer initiation, promotion, and progression in differentiated cells.

PS 1020 MICRO ELECTRODE CHIP ASSAY (MEA) AS METHOD TO DETECT NEUROTOXICITY *IN VITRO.*

S. Vogel[1], A. Novellino[2], E. De Franchi[2], B. van Ravenzwaay[1] and R. Landsiedel[1]. *[1]BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany and [2]ETTs.r.l, Genova, Italy.*

Detection and characterization of chemical-induced toxic effects in the nervous system represent a major challenge for registration and assessment of chemicals. So far, no in vitro method for evaluating the neurotoxic hazard has yet been validated and

accepted for regulatory purpose. In vivo, neurotoxicological assessments exploit the fact that activity of neurons in the central and peripheral nervous system has functional consequences. The microelectrode array (MEA) assay consists of a culture chamber into which an integrated array of microelectrodes is capable of measuring extracellular electrophysiology (spikes and bursts) from electro-active tissues. A wide variety of electrically excitable biological tissues may be placed onto the chips including primary cultures of nervous system tissue. Recordings from such an in vitro cultured system are non invasive, label free evaluations and provide a higher throughput than conventional electrophysiological techniques. In this fist study 21 blinded substances were tested in a dose-response curve on embryonic rat cortical neuronal networks on a MEA for their toxicity. The experimental procedure consisted in the evaluation of the firing activity (spiking rate) and modification/reduction in response to the chemical administration. Native/reference activity, 30 minutes of activity recording per dilution, plus the reversibility/recovery points (after 24 hours) were recorded. The IC50 values were calculated using Hill Equation Fitting tool of the averaged data. The preliminary data, using a set of chemicals with different mode-of-actions (12 known to be neurotoxic, 2 non neuroactive and not toxic and 7 not neuroactive but toxic) show a good predictivity (sensitivity: 0.93; specificity: 0.57; predictivity: 0.81). Thus, the MEA with a neuronal network has the potency to become a powerful tool to evaluate the neurotoxicity of substances in vitro.

PS 1021 EFFECT OF METALS ON β-ACTIN AND TOTAL PROTEIN SYNTHESIS IN CULTURED HUMAN INTESTINAL EPITHELIAL CELLS.

A. R. Calabro and F. A. Barile. *Pharmaceutical Sciences, St. John's University College of Pharmacy, Queens, NY.*

As an important structural protein, β-actin is associated with anchoring of tight junctions (TJs) to the cell scaffold. Caco-2 cells, an immortal intestinal epithelial cell line, rely on β-actin to form intact monolayers with high transepithelial electrical resistance in cell culture inserts. We examined the effect of six metals on expression of β-actin mRNA and β-actin synthesis, on total and net production of newly synthesized proteins, and on paracellular permeability (PP) of TJ markers, in confluent monolayers of Caco-2 cells. [^{3}H]-glycine and [^{3}H]-tyrosine were used as indicators of newly synthesized proteins in the absence or presence of increasing concentrations of arsenic, cadmium, copper, manganese, mercury and nickel. Monolayers were exposed to each metal for 24-hr as well as continuous daily repeated exposures for 48- and 96-hr. Results suggest that decreases in newly synthesized proteins, in which β-actin represents about 10%, correlated with 2- to 5- fold higher expression of β-actin mRNA for the higher concentrations of metals. Interestingly, IC_{50}s calculated for each chemical for 24-hr acute and 48- and 96-hr repeated dosing experiments, using the MTT viability assay and PP markers, decreased newly synthesized and total proteins to 10% and 40% of control, respectively. Overall, the results indicate that, at equivalent concentrations, the metals affect β-actin mRNA and newly synthesized proteins before cell viability and PP are compromised. Consequently, the data contributes to elucidating the mechanisms of metal cytotoxicity that lead to understanding the relationship between TJ integrity, paracellular transport, and cell viability. (Supported in part by a grant from the International Foundation for Ethical Research, Chicago, IL; No. 35262-985).

PS 1022 FETAL PHTHALATE SCREEN: ASSESSMENT OF SEVERAL PHTHALATE ESTERS (PE) ON FETAL RODENT TESTOSTERONE (T) PRODUCTION AND GENE EXPRESSION FOLLOWING *IN UTERO* EXPOSURE.

C. Lambright[1], J. Furr[1], M. Cardon[1], B. Hannas[2], D. Bermudez[5], N. Wrench[3], V. Wilson[1], P. Foster[4] and E. Gray, Jr[1]. *[1]ORD/TAD, U.S. EPA, Durham, NC, [2]NRC Postdoctoral Fellow, Durham, NC, [3]Student Contractor, Durham, NC, [4]NTP, NIEHS, Durham, NC and [5]NC State Postdoctoral, U.S. EPA, Durham, NC.*

. PE are a large family of compounds used in a wide array of consumer, industrial and medical products. Studies have shown that in utero treatment with PE such as diethyl hexyl phthalate (DEHP) during the critical period of fetal reproductive development produced male reproductive malformations by reducing fetal T production and gene expression. It has been proposed that PE with straight side chains of C4 to C6 are likely reproductive toxicants but C3 or shorter and C7 or longer are not. The goal of this study was to test this by using a relatively rapid in vivo screen to evaluate a suite of PE for their potential reproductive toxicities. We investigated the effects of 12 PE on fetal testes T production and gene expression by exposing time pregnant Sprague-Dawley rats via oral gavage (750 mg/kg/day) from gestational day (GD) 14-18. On GD 18, testes from three fetuses were collected and cultured for 3 hours. Medium was collected and T levels measured by RIA. The remaining testes were pooled by litter, mRNA extracted and gene expression for Insl3, STAR and Cyp11a was measured. Fetal testes T production was significantly reduced compared to control following treatment with BBP, DBP, DIBP, DPP, DiHP, DHeP(diheptyl-), DHP (dihexyl-), DCHP (dicyclo-), and DINP. No effect on fetal T was seen with DEP, BrDEHP, DOTP or DiNCH. Gene expression of Insl3, STAR and Cyp11a was significantly reduced as compared to control by some of the above PE that also reduced T production. Some PE reduced reduce both fetal testes T production and gene expression with the reduction in T production being consistently the most robust response. Furthermore, some PE with straight chains less than C4 or longer than C6 disrupted fetal T synthesis. Disclaimer: This abstract doesn't reflect USEPA policy. Supported in part by NTP/NIEHS IA# RW7592285501-1

PS 1023 DETERMINING ESTROGENIC ACTIVITY IN SERUM FROM OVARIECTOMIZED RATS TREATED WITH ENVIRONMENTAL COMPOUNDS USING AN *IN VITRO* ESTROGEN-MEDIATED TRANSCRIPTIONAL ACTIVATION ASSAY (T47D-KBLUC).

N. Wrench[1, 3], L. Gray[1], D. S. Bermudez[2], J. Furr[1] and V. S. Wilson[1]. *[1]NHEERL, TAD, U.S. EPA, Durham, NC, [2]Postdoctoral Fellow, North Carolina State University, Raleigh, NC and [3]Student Contractor, U.S. EPA, Durham, NC.*

The use of cell-based assays to quantify low levels of estrogen in human serum is an accepted method. These assays are more sensitive but less specific than radioimmunoassays (RIA). Thus, we hypothesized that estrogen responsive T47D-KBluc cells would detect estrogenic activity in serum from ovariectomized (ovx) rats dosed with estradiol as well as other estrogenic compounds such as bisphenol A-F (BPAF). Ovx rats were dosed subcutaneously with a negative control (corn oil), positive control (1 ug/day estradiol-benzoate; EB) or BPAF (600mg/kg/d) for 3 consecutive days. At necropsy, blood was collected, centrifuged and the serum was stored at -80°C. Cells were exposed to rat serum diluted 100-fold in RPMI media, supplemented with 5% dextran-coated-charcoal stripped fetal bovine serum, at 100ul/well in triplicate. Negative control serum did not induce estrogenic activity. Dilutions of the rat serum demonstrated a dose responsive decline in estrogenic activity for both EB and BPAF. Calculated estrogen equivalence (EEQ) values for the EB samples (run in duplicate) averaged: 1.16ng/ml, 0.37ng/ml, 0.22ng/ml. RIA results for the EB samples of: 1.78ng/ml, 0.53ng/ml, 0.30ng/ml, respectively, confirmed the accuracy of the cell assay results. Calculated BPAF equivalents in the rat serum were: 12861ng/ml, 5092ng/ml, 6951ng/ml. These results demonstrate that the estrogen-mediated T47D-KBluc cell assay can detect circulating estradiol levels with sensitivity comparable to an RIA, and can be used to quantify estrogenic activity in serum from rats treated with BPAF. Disclaimer: This abstract does not necessarily reflect EPA policy.

PS 1024 REPRODUCTIVE ENDPOINTS OF FEMALE WISTAR RATS EXPOSED TO TESTOSTERONE *IN UTERO* AND DURING LACTATION.

M. T. Guerra[1], R. F. Silva[2], M. Sanabria[2], A. C. Gaspar[2] and W. G. Kempinas[2]. *[1]Unicamp, Campinas, São Paulo, Brazil and [2]Morphology, Unesp, Botucatu, São Paulo, Brazil.* Sponsor: S. Cohen.

Female reproductive disorders have increased in recent years and exposure to environmental chemicals may contribute to several gynecologic pathologies, especially when it occurs during critical periods of development. Despite some concern since the 1970s, the environmental presence of androgens from anthropogenic origin was only confirmed within the last few years, and compounds with androgenic activity were found as contaminants in rivers and in animals intended for human feeding. In humans, the effects of exposure to androgens on development following medical conditions, such as polycystic ovary syndrome or congenital adrenal hyperplasia, are still to be studied. The aim of this work was to evaluate reproductive parameters of female offspring exposed to testosterone propionate (TP) in utero and during lactation. Pregnant Wistar rats received either corn oil (GI/n=8) or TP at concentrations of 0.05 mg/kg (GII/n=7), 0.1 mg/kg (GIII/n=9) or 0.2mg/kg (GIV/n=10), s.c., from gestational day (GD) 12 to post-natal day (PND) 21. The following parameters were assessed: body weight, anogenital distance (AGD), nipple counting, external signs of puberty onset (age of vaginal opening and first estrus), uterus, ovaries, liver, kidneys and pituitary weights, estrous cycle and sexual behavior. Results showed no effect upon body weight (except for a decrease at PND22 and after puberty onset in the GIV group), AGD was only increased in GIV group at PND1, nipple counting and puberty onset were similar among groups, the weight of the kidneys was only impaired in GIV group at PND75, the

estrous cycle and sexual behavior did not show any changes due to treatment. Based on these results, we conclude that exposure to TP in utero and during lactation did not significantly impair reproductive endpoints of female offspring.
Financial support: CNPq, CAPES, FAPESP

PS 1025 EFFECTS OF PREIMPLANTATION BISPHENOL-A EXPOSURE ON EMBRYO TRANSPORT, EMBRYO IMPLANTATION, AND POSTNATAL BODY WEIGHT IN C57BL6 MICE.

X. Ye, S. Xiao, H. Diao and F. Zhao. *University of Georgia, Athens, GA.*

Bisphenol A (BPA) is an endocrine disruptor widely used in polycarbonate plastics and epoxy resins. It has been reported that BPA has adverse effects on uterine development; it alters uterine expression of progesterone and estrogen receptors; and BPA at ~400 mg/kg is detrimental to embryo implantation. Embryo implantation is a synchronized process between a competent embryo and a receptive uterus. Uterine receptivity is a hormone-controlled transient state in which the uterus can accept an embryo to implant. The mechanism of BPA in disrupting embryo implantation is currently unknown. To investigate the potential effects of BPA on preimplantation embryos and uterine receptivity, timed pregnant wild type C57BL6 young adult females were treated with 0, 10, 40, 100, and 400 mg/kg BPA subcutaneously daily from gestation day 0.5 to day 3.5, and detected for implantation sites on day 4.5. Embryo implantation appeared normal in 10 mg/kg BPA-treated mice, delayed implantation was detected in 40 mg/kg BPA-treated uteri, no implantation was detected in 100 and 400 mg/kg BPA-treated uteri, indicating defective embryo implantation in 40, 100, and 400 mg/kg BPA-treated mice. Progesterone receptor (PR), which disappears from uterine luminal epithelium (LE) upon implantation, remained highly expressed in the LE of day 4.5 uteri from 40, 100, and 400 mg/kg BPA-treated mice, further demonstrating defective embryo implantation in these BPA-treated mice. It is currently being investigated about the involvement of embryo and uterus on the defective embryo implantation upon BPA treatment. Available preliminary data suggest delayed transport of embryos to the uterus in 40 mg/kg BPA-treated mice and defective uterine receptivity in 100 mg/kg BPA-treated mice. Subcutaneous 40 mg/kg BPA exposure during preimplantation also led to prolonged gestation period, increased perinatal lethality, and increased postnatal bodyweight of the pups. These data demonstrate that BPA exposure during preimplantation can affect embryo transport, embryo implantation, and postnatal body weight of the offspring.

PS 1026 CIGARETTE SMOKE EXPOSURE RESULTS IN SIGNIFICANT FOLLICLE LOSS *VIA* AN ALTERNATIVE OVARIAN CELL DEATH PATHWAY.

A. M. Mulligan Tuttle[1, 2], M. Stämpfli[3] and W. G. Foster[2]. [1]*Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada,* [2]*Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada and* [3]*Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada.*

Delayed conception, decreased success in assisted reproductive technologies and premature ovarian failure have all been reported in female smokers compared with non-smokers. Although cigarette smoking in humans and cigarette smoke exposure in mice depletes the primordial follicle population, the mechanism of action is unknown. Therefore, the effect of cigarette smoke exposure, at concentrations representative of human exposure, on selective stage-dependent destruction of primordial and primary follicles in mouse ovaries was studied. Recently we reported that an 8 week exposure to cigarette smoke exposure resulted in significant primordial follicle loss that was not that was not associated with activation of either the intrinsic or extrinsic apoptosis pathways as shown by absence of an effect on TUNEL staining, DNA laddering, and activated caspase-3 expression compared to controls. However, a significant decrease in the expression of the inhibitor of apoptosis B-cell lymphoma-2 (Bcl-2) in ovaries of cigarette smoke exposed mice vs. controls was found. Bcl-2 is a key regulatory protein common to both apoptosis and autophagy (self-digestion) pathways that inhibits cell death via blocking of Bax in apoptosis and Beclin-1 in autophagy. In this study, mice were exposed to CS or room air control for 8 weeks. Ovaries were excised and processed for electron microscopy (EM), Western blotting, immunohistochemistry (IHC) and quantitative real time-PCR (q-PCR). In this study, we found an increase in markers of autophagy, including an increase in Lox-I and LC3 expression and an increase in the presence of autophagosomes in the granulosa cells of smoke exposed ovaries. Taken together, our results suggest that CS exposure depletes the ovarian follicle reserve through a decrease in Bcl-2 expression leading to activation of the autophagy pathway, a novel alternative ovarian cell death pathway that is largely unexplored in the ovary.

PS 1027 ASSESSING POTENTIAL NON-SPECIFIC IMMUNE-MEDIATED EFFECTS IN REPRODUCTIVE TOXICITY STUDIES WITH VACCINES.

L. Segal[1], D. Stannard[2], D. Myers[2], D. Morelle[1], S. Veenstra[1] and B. Baras[1]. [1]*GlaxoSmithKline Biologicals, Rixensart/Wavre, Belgium and* [2]*Huntingdon Life Sciences, Eye, Suffolk, United Kingdom.*

The design of regulatory reproductive/fertility studies for vaccines are not prescribed. During the H1N1 Flu pandemic pregnant women were considered a high risk group for H1N1 infection and were a priority group in vaccination programs. GSK Biologicals' AS03-adjuvanted pandemic split influenza vaccines were used in vaccination campaigns worldwide and were tested in rat pre-, peri- and post-natal reproductive toxicity studies prior to licensure. In these studies, rats were treated with saline, AS03 or the pandemic influenza vaccine. The dosing schedule included pre-mating exposure, injections on gestation days 6, 8, 11 and 15, and a final dose on lactation day 7 for dams allocated to litter. Although these previously-conducted studies demonstrated a strong and sustained specific immune response during the pre-implantation phase, the study design was deemed deficient by the Regulators for the evaluation of non-specific immune responses on implantation and early pregnancy loss, as no treatment was given during this period. Consequently, GSK Biologicals performed a post-registration study in which pregnant rats were dosed daily from gestation day 0-6 with saline, AS03 or H1N1v/AS03, to determine if the non-specific immune response induced by AS03, alone or in combination with the H1N1v antigen, affected embryo implantation. Daily intramuscular injection of H1N1v/AS03 or AS03 produced signs of mild maternal toxicity and in conjunction with the serology data was considered to provide clear evidence that the dams were systemically exposed to H1N1v/AS03 or AS03 during early gestation. Nonetheless, no adverse effects on pregnancy status, litter size or pre- or post-implantation losses were seen. In order to ensure that future studies include an evaluation of non-specific immune responses on implantation and early pregnancy loss, GSK collaborated with HLS in re-designing the gestation period dosing schedule to include intramuscular injections to dams on gestation days 3 (instead of 6), 8, 11 and 15.

PS 1028 COMPARISON OF THE REPRODUCTIVE PERFORMANCE OF THE HARLAN AND CHARLES RIVER SPRAGUE-DAWLEY RAT.

E. Mylchreest and K. K. Daniels. *Southern Research, Birmingham, AL.*

As part of developing reproductive toxicology testing capabilities at our facility, pilot studies were conducted to generate historical control data for the Sprague-Dawley rat from Charles River (Crl:CD) and Harlan (Hsd:SD). Groups of nulliparous rats were mated in-house or obtained timed mated from each vendor (n=25 to 30 per group). Dams were allowed to deliver and rear their offspring until weaning. The number of live and dead, individual body weights, and clinical observations were determined for pups at postnatal day (PND) 0, 1, 4, 7, 14, and 21. Litters were culled to 4/sex (litter size permitting) on PND 4. Fertility was lower in the Hsd:SD rat in both in-house mated (80%) and timed mated (83%) compared to the timed mated Crl:CD rat (100%). The timed mated Hsd:SD rat had smaller litters than the Crl:CD rat (average of 10.6 vs 12.5 pups/litter on PND 0) and correspondingly fewer uterine implantation sites, but similar postimplantation loss. The percent pups born alive, Day 0-1 viability, and Day 4-21 survival were 92%, 95% and 95% in the timed mated Hsd:SD rat, respectively, compared to >99% for all three indices in the Crl:CD rat. The in-house mated Hsd:SD rats had high postimplantation loss (26%), low pup viability/survival (84% to 86%), and low litter survival to PND 21 (78%) compared to their timed mated counterparts (92%) and the Crl:CD rats (100%). The high pup mortality together with poor nest building, pups not nursing, and cannibalization of pups, suggests poor maternal behavior in the Hsd:SD dams. Although fertility and litter viability/survival were not optimal in any of the Hsd:SD groups evaluated, the rats in the timed mated group performed slightly better those that were mated in-house. In conclusion, these preliminary data indicate that, under the same environmental conditions at our facility, the reproductive performance of the Crl:CD rat is optimal and superior to the Hsd:SD rat.

PS 1029 DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF ISODECYL BENZOATE.

K. L. Pavkov[1] and E. D. Sloter[2]. [1]*Department of Toxicology and Environmental Sciences, ExxonMobil Biomedical Sciences, Inc., Annandale, NJ and* [2]*Developmental and Reproductive Toxicology, WIL Research Laboratories, LLC., Ashland, OH.*

Isodecyl benzoate (IDB; CASRN 131298-44-7) is a C10 alkyl benzyl ester. Studies of the potential for IDB to produce developmental or reproductive toxicity were conducted in Sprague-Dawley rats. In the developmental toxicity study female rats

were given IDB at dosages of 0, 30, 300, or 1000 mg/kg by oral gavage on a daily basis from GD 6 through 15. Effects in the 1000 mg/kg dose group included a transient body weight (bwt) loss for the dams, a slight decrease in mean fetal bwt, and a reduction in the incidence of cervical centrum #1 ossification. This study showed that IDB does not cause any significant developmental or maternal effects and the NOAEL for the minor effects observed was 300 mg/kg/day.
In the two-generation reproductive toxicity study, IDB was given by dietary administration at 0, 1000, 3000, or 10000 ppm. There were no effects on reproductive performance (mating, fertility, copulation, and/or conception indices) or estrous cyclicity and no differences in spermatogenic parameters. Accordingly, the reproductive NOAEL was the highest dose tested of 10,000 ppm (equivalent to ~ 676 mg/kg/day for males and 641-1469 mg/kg/day for females). The NOAEL for minor offspring effects was 3000 ppm (equivalent to parental doses of ~195 mg/kg/day for males and 191-440 mg/kg/day for females) based on lower birth weights (PND 1) in the F1 and F2 offspring (10000 ppm) with lower bwt in the pre-weaning period. There were no effects on areola retention (male offspring) or ano-genital distance. Balanopreputial separation was delayed in the offspring from the 10000 ppm group, most likely due to the lower body weights in this group. These data showed that IDB does not influence endocrine-mediated reproductive processes. The overall conclusion from these studies was that IDB does not cause any significant developmental or reproductive effects.

PS 1030 EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY TEST WITH LEAD ACETATE.

R. Kubaszky[1], D. J. Esdaile[1], T. Hanley[2], P. Maslej[1], D. Minnema[2], J. Wright[3] and R. Lewis[3]. *[1]Lab. Research Ltd., Veszprem, Hungary, [2]Syngenta Crop Protection, LLC, Greensboro, NC and [3]Syngenta, Jealotts Hill, Bracknell, United Kingdom.*

This study was designed to demonstrate the utility of an extended one-generation reproduction study in rats as proposed by draft OECD guideline and the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI) and by Cooper, et al (2006). Lead Acetate was provided in drinking water to the F0 adult Wistar rats throughout pre-mating, gestation, and lactation, and to the F1 generation to adulthood. The adults (24 rats/sex/group) were given concentrations of 0 (sodium acetate control), 100, 800 and 1700 ppm Pb, beginning 2 to 4 weeks prior to mating. Adults were mated and evaluated for standard in-life parameters, reproductive evaluations and histopathology. Resulting litters were culled and then assigned to 3 subgroups for clinical pathology/thyroid function/neurotoxicity, immunotoxicity and reproductive toxicity assessments. Selected pups were evaluated for developmental milestones, growth, neurobehavior assessments (FOB, grip strength, landing foot splay, motor activity), TDAR (sRBC) immunotoxicity, thyroid hormones, standard pathology and neurohistopathology.
Dose-related decreases in water consumption were observed in F0 adults and F1 offspring at all concentrations. Decreases in sperm parameters (sperm counts, motility, and morphology) were observed in the F0 and F1 males. At the high dose, effects on early post-natal survival, lower pup body weights throughout lactation, decreases in anogenital distance, and delayed vaginal opening and preputial separation were observed. Although not statistically significant from control, slightly lower serum IgM levels were noted for the 1700 ppm males. Despite minimal evidence of neurobehavioral changes, neuropathological examination indicates a treatment-related peripheral neuropathology.
This outcome illustrates the utility of using an integrated study design to investigate the various aspects of developmental neurotoxicity, immunotoxocity, and reprotoxicity.

PS 1031 DISTRIBUTION OF ATRAZINE (ATR) AND METABOLITES IN THE WISTAR RAT FOLLOWING GESTATIONAL/LACTATIONAL EXPOSURES.

T. E. Stoker[1], A. Kamel[2], Y. Qian[2], L. Podhorniak[2], R. Cooper[1] and L. Strader[1]. *[1]Endocrinology Branch, U.S. EPA, Research Triangle Park, NC and [2]Analytical Chemistry Branch, U.S. EPA, Ft. Meade, DC.*

Gestational/lactational exposure to ATR is reported to alter reproductive/developmental function, yet our understanding of the transfer of ATR and/or its metabolites from the dam to the fetus/offspring is limited. Previously we examined the lactational transfer of C14-ATR, but specific ATR metabolites were not determined. In the current study, the distribution of atrazine and certain metabolites were determined in the dam, fetus and neonate following gavage exposure. Groups of dams were exposed to 0, 5 and 25 mg/kg ATR from (1) gestation day (GD) 18-20, (2) GD 14-20 or (3) GD 14- postnatal day (PND) 10. Two hours after the last dose, blood was drawn by cardiac puncture and the dam perfused with saline. GD20 fetuses were collected, along with the plasma, brain, adrenal glands and mammary glands of the dam. On PND 10 pups were separated from the dam for 2.5 h and then allowed to nurse 30 minutes before plasma, brain, adrenal and milk samples were collected. We measured ATR, desethyl ATR (DEA), desisopropyl ATR (DIA), diaminochlorotriazine (DACT), and hydroxyl ATR (OH-ATR) using LC/MS/MS. In the dams DACT accounted for the highest concentration in plasma and tissues, followed by DIA and DEA, with similar amounts present after both the 3 and 7 day exposure. ATR was present in very low amounts. OH-ATR was not detected in the majority of the samples. There was transfer of ATR from the dam to the fetus on GD 20 following both exposures and doses. Although all analytes were present in the GD20 fetus, DACT contributed to over 75% of the total. In the PND10 pups DACT accounted for 99% of the analytes which correlated well with the 91% of DACT found in the milk. This study demonstrates that DACT was the primary metabolite found in the dam and fetus/neonate at 2 h after the last dose and that ATR crossed both the placental and blood-brain barrier of the dam/neonate. This study does not reflect EPA policy.

PS 1032 LOW-DOSE, GESTATIONAL EXPOSURE TO ATRAZINE DOES NOT ALTER POSTNATAL REPRODUCTIVE DEVELOPMENT OF MALE OFFSPRING.

M. J. Fraites, D. S. Best, M. G. Narotsky and R. L. Cooper. *Toxicology Assessment Division, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC.*

There is growing evidence that xenobiotic exposure during the perinatal period may result in a variety of adverse outcomes when the developing organism attains adulthood. Maternal stress and subsequent exposure of the fetus to excess glucocorticoids may underlie these effects. Previous studies have demonstrated that high gestational doses of the chlorotriazine herbicide, atrazine (ATR), alter reproductive development of the male rat. ATR also acutely increases circulating glucocorticoids in adult rats. Therefore, the current studies investigated the development of male Sprague Dawley rat offspring following 1) lower doses of ATR during gestation and 2) an ATR dosing regimen designed to increase daily maternal glucocorticoid exposure. Timed-pregnant dams were administered 1, 5, 20, or 100 mg/kg/d ATR from gestation day 14–21 in a single oral gavage dose once per day (s.i.d) or the same dosage of ATR split into two doses per day (b.i.d.) to elevate glucocorticoid levels during the nadir and peak of the diurnal rhythm. Lower doses of ATR (1–20 mg/kg/d) administered s.i.d. or b.i.d. did not alter offspring body weight, neonatal testosterone production, timing of puberty, anogenital distance, adult testosterone or luteinizing hormone levels, or adult androgen-sensitive tissue weights. A similar level of maternal toxicity was observed at 100 mg/kg/d in both studies; however, this dosage administered b.i.d. resulted in significantly higher mortality of offspring after birth. Although birth weight was significantly reduced in high-dose litters of both studies (and remained lower through PND 59 in s.i.d. offspring), the reproductive development of these offspring was not altered. We conclude that gestational exposure to ATR at lower dosages (≤20 mg/kg/d) has no significant effect on pup survival or the male developmental endpoints assessed in these studies. Significant offspring mortality may have precluded observations of altered development in high-dose litters. This abstract does not necessarily reflect EPA policy.

PS 1033 BISPHENOL A INDUCES ATRESIA IN MOUSE ANTRAL FOLLICLES.

J. Peretz, R. K. Gupta and J. A. Flaws. *Comparative Biosciences, University of Illinois Urbana-Champaigne, Urbana, IL.*

Bisphenol A (BPA) is a prominent chemical in the manufacturing of plastics and consumer goods. In a government funded human study (NHANES), BPA, with a half-life of at least 4-6 hours, was present in 95% of random urine samples, indicating that humans are constantly exposed to this chemical. Further, BPA has been found to cause adverse effects in numerous tissues throughout the human body, including ovarian antral follicles. Previous studies in our lab have shown that exposure to BPA inhibits antral follicle growth and steroidogenesis in the adult mouse ovary. However, the health of these BPA-treated antral follicles has not been evaluated. Thus, this study tested the hypothesis that BPA exposure (4.4μM to 440μM) induces atresia in antral follicles. To test this hypothesis, antral follicles were isolated from adult mice on postnatal day (PND) 32 and cultured continuously with either vehicle (dimethylsulfoxide; DMSO) or BPA (4.4, 44, or 440μM) in supplemented α–minimum essential media for 120h. After culture, the follicles were evaluated for atresia. Further, RNA was isolated from follicles, reverse transcribed into cDNA, and subjected to real-time PCR for various genes known to regulate atresia. The results indicate that BPA (440μM) significantly induced atresia in the antral follicles (DMSO: 1.3±0.15; BPA440μM: 4.0±0.0), significantly increased mRNA expression levels of the anti-apoptotic factor B-cell lymphoma 2 (Bcl2) (DMSO: 0.08±0.01; BPA440μM: 1.5±0.46ge; genomic equivalents (ge);n=3;p=0.02) and significantly decreased mRNA expression of the antioxidant superoxide dismutase (SOD) (DMSO: 0.27±0.15; BPA440μM: 0.07±0.33 ge;n=3;p=0.02) compared to DMSO controls. BPA exposure did not affect Bcl2-associated X protein or catalase

mRNA expression levels. Collectively, these data indicate that BPA causes atresia and alters some antioxidant factors in antral follicles. Support: NIHR01 ES019178, P20ES018163.

PS 1034 EVALUATION OF A COMBINED DERMAL DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PEA (PHENETHYL ALCOHOL) IN RATS—INCLUDING AN EXAMINATION SKELETAL VARIANTS POSTNATALLY.

V. T. Politano[1], E. M. Lewis[2], A. M. Hoberman[2], R. M. Diener[3] and A. Api[1]. *[1]Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ, [2]Charles River Laboratories Preclinical Services, Horsham, PA and [3]Argus International, Inc., Horsham, PA.*

The purpose of this study was to determine the reversibility of fetal skeletal alterations and delays in skeletal ossification following percutaneous, occluded application of neat doses of 140, 430 or 1400 mg/kg/day Phenethyl Alcohol (PEA) to four groups of 40 Crl:CD(SD) female rats on Gestation Days 7 through 20 (GDs 7 through 20). Twenty rats in each group were Caesarean-sectioned, while the remaining 20 rats in each group were allowed to deliver their litters. The highest dosage, 1400 mg/kg/day, was maternally and developmentally toxic. The developmental No-Observable-Effect-Level (NOEL) for Caesarean-delivered fetuses on GD 21 was 140 mg/kg/day. Higher dosages caused reductions in fetal weight and delays in skeletal ossification. The No-Observable-Adverse-Effect-Level (NOAEL) in the naturally delivered pups at Postpartum Day 21 (PPD 21) was 430 mg/kg/day. All delays in ossification and increased incidences of cervical ribs at the 7th cervical vertebrae observed on GD 21 in the Caesarean-delivered fetuses at 430 mg/kg/day were resolved by PPD 21 in the naturally delivered pups at 430 mg/kg/day.

PS 1035 PREGNENOLONE CO-TREATMENT DOES NOT RESTORE GROWTH OF MOUSE ANTRAL FOLLICLES TREATED *IN VITRO* WITH THE MONO-HYDROXYLATED METABOLITE OF METHOXYCHLOR.

Z. R. Craig, P. R. Hannon and J. A. Flaws. *Comparative Biosciences, University of Illinois, Urbana, IL.*

Antral follicles are the functional units of the ovary and are responsible for ovarian steroidogenesis and ovulation of oocytes for fertilization. Chemicals that disrupt antral follicle function have the potential to lead to infertility. Mono-OH methoxychlor (mono-OH) is a metabolite of the model endocrine disruptor, methoxychlor (MXC). Mono-OH has been shown to inhibit growth, increase follicular death, and decrease steroidogenesis by cultured mouse antral follicles. Because synthesis of pregnenolone is considered the rate-limiting step in ovarian steroidogenesis, we hypothesized that co-treating antral follicles with pregnenolone would prevent mono-OH induced inhibition of follicle growth. To test this hypothesis, antral follicles isolated from female CD-1 mice (32-35 days old) were either untreated or exposed in vitro to dimethylsulfoxide (DMSO, vehicle control), or mono-OH (10 μg/mL) with and without pregnenolone (1 μg/mL) for 96 hours. Follicle diameters were monitored every 24 h and used to measure the rate of follicular growth (% change) over the 96-h culture period. In agreement with previous reports, antral follicles treated with mono-OH did not grow during the 96-h culture period when compared to DMSO-treated follicles (DMSO: 36.24 ± 6.7 % change; mono-OH: 6.10 ± 1.8 % change; $P \leq 0.05$, n=3). Co-treatment of antral follicles with pregnenolone did not prevent inhibition of growth in antral follicles treated with mono-OH (-6.84 ± 1.8 % change; $P > 0.05$; n=3). Finally, pregnenolone treatment did not affect growth of follicles treated with DMSO vehicle (30.93 ± 2.7 % change; $P > 0.05$). Collectively, these results suggest that mono-OH potentially inhibits follicular growth by a mechanism independent of availability of pregnenolone for steroidogenesis. Funded by NIH R01 ES012893, ES019178 and Billie A. Field Fellowship in Reproductive Physiology.

PS 1036 DI-(2-ETHYLHEXYL) PHTHALATE AND MONO-(2-ETHYLHEXYL) PHTHALATE INHIBIT GROWTH OF OVARIAN ANTRAL FOLLICLES THROUGH AN OXIDATIVE STRESS PATHWAY.

W. Wang, Z. R. Craig, M. S. Basavarajappa, R. K. Gupta and J. A. Flaws. *Comparative Bioscience, University of Illinois, Urbana, Urbana, IL.*

Phthalates are synthetic plasticizers used widely in plastics and many other common consumer products. Exposure to these chemicals can cause developmental and reproductive toxicity. Our previous studies show that di-(2-ethylhexyl) phthalate (DEHP) and its active metabolite, mono-(2-ethylhexyl) phthalate (MEHP), can inhibit follicle growth and cause atresia of antral follicles. However, the mechanisms by which DEHP and MEHP inhibit growth and cause atresia are not fully understood. Oxidative stress has been linked to phthalate-induced toxicity in non-reproductive tissues. Thus, we tested the hypothesis that DEHP and MEHP inhibit the growth of antral follicles through an oxidative stress pathway. Antral follicles were isolated from ovaries of adult CD-1 mice (age 30-35 days) and cultured with vehicle control (dimethylsulfoxide [DMSO]), DEHP (1-100μg/ml) or MEHP (0.1-10μg/ml) ± N-acetyl cysteine (NAC, an antioxidant) (0.5-2mM) for 96h. During culture, follicle size was evaluated daily as a measurement of follicle growth. At the end of culture, follicles were subjected to measurement of the expression of various key antioxidant enzymes: Cu/Zn superoxide dismutase (SOD1), glutathione peroxidase (GPX), and catalase (CAT) by quantitative real-time PCR. The results indicate that DEHP (1-100μg/ml) and MEHP (1-10μg/ml) inhibited the growth of follicles compared to DMSO controls and that NAC (0.5-2mM) blocked the ability of DEHP and MEHP to inhibit follicle growth. DEHP and MEHP reduced the expression of SOD1, whereas NAC blocked the effects of DEHP and MEHP on SOD1 expression. However, the expression of GPX and CAT were not affected by the treatments. Collectively, these data suggest that DEHP and its active metabolite, MEHP, inhibit follicle growth by inducing oxidative stress. (Supported by R01 ES 019178)

PS 1037 METHOXYCHLOR ALTERS STEROIDOGENIC ENZYMES IN CULTURED MOUSE ANTRAL FOLLICLES.

M. Basavarajappa, Z. Craig, B. Karman, W. Wang and J. Flaws. *Comparative Biosciences, University of Illinois, Urbana, IL.*

The organochlorine pesticide methoxychlor (MXC) is a known endocrine disruptor that affects adult female rodents by causing reduced fertility, persistent estrus, and ovarian atrophy. MXC is widely used in many countries to prevent and kill the various species of insects that attack crops, vegetables, fruits, and animals. Previously, we demonstrated that MXC reduces estradiol, testosterone, and androstenedione levels in antral follicles. The goal of the present study was to determine whether MXC reduces estradiol and its biosynthetic hormone intermediates by altering steroidogenic enzymes present in the antral follicles of the ovary. To accomplish this goal, antral follicles were mechanically isolated from ovaries of CD1 female mice aged 35-40 days. The isolated antral follicles (10-15 per treatment) were cultured in supplemented α-minimum essential media in the presence of dimethylsulfoxide (control; DMSO) or MXC (1, 10, 100 μg/ml) for 96 h at 37°C and 5%CO2. At the end of the culture, follicles were snap frozen, RNA was isolated, cDNA was synthesized, and quantitative real time PCR was performed to measure the levels of steroidogenic enzymes present in antral follicles. Results were expressed as a ratio of starting quantity (SQ) of each gene mRNA to β-actin (Actn). The data indicate that MXC significantly decreases the expression of steroid acute regulatory protein (Star; DMSO=1.57±0.32, MXC10=0.31±0.03, MXC100=0.70±0.12 SQ; $p<0.05$, n=3), cholesterol side-chain cleavage (Cyp11a1; DMSO=1.10±0.16, MXC10=0.40±0.05, MXC100=0.19±0.03 SQ; $p<0.05$, n=3), 3β hydroxysteroid dehydrogenase 1 (Hsd3b1; DMSO=0.65±0.02, MXC10=0.52±0.01, MXC100=0.43±0.05 SQ; $p<0.05$, n=3), 17β-hydroxysteroid dehydrogenase 1 (Hsd17b1; DMSO=0.61±0.14, MXC10=0.77±0.06, MXC100=0.22±0.03 SQ; $p<0.05$, n=3), and aromatase (Cyp19a1; DMSO=0.57±0.04, MXC10=0.20±0.03, MXC100=0.012±0.001 SQ; $p<0.05$, n=3) in antral follicles. Thus, these data suggest that MXC reduces estradiol and its biosynthetic hormone intermediates in antral follicles by decreasing the expression of steroidogenic enzymes. NIH R01 ES09178

PS 1038 ESTROGEN RECEPTOR ALPHA OVEREXPRESSING MOUSE OVARIES MAY BE SUSCEPTIBLE TO METHOXYCHLOR AND ITS METABOLITES BECAUSE OF REDUCED METABOLISM OF THE CHEMICALS.

T. Paulose, I. Hernández-Ochoa, M. S. Basavarajappa, J. Peretz, Z. R. Craig and J. A. Flaws. *Comparative Biosciences, University of Illinois Urbana-Champaign, Urbana, IL.*

Methoxychlor (MXC) is an organochlorine pesticide that was used worldwide on crops, fruits and vegetables. MXC causes adverse ovarian toxicity including inhibition of antral follicle growth. Since MXC binds to estrogen receptor alpha (ESR1), we hypothesized that ovaries that overexpress ESR1 will be more susceptible to toxicity induced by MXC and its metabolites, mono-OH MXC (MOH) and HPTE. To test this hypothesis, we generated and validated a transgenic mouse model in which ESR1 is overexpressed (ESR1 OE) in the ovaries. We cultured antral follicles from controls and ESR1 OE mice with either the vehicle dimethylsulfoxide (DMSO), MXC (1-100 μg/ml), MOH or HPTE (0.1-10 μg/ml). The growth of

the antral follicles was monitored every 24h for a period of 96h. Further, we examined the gene expression levels of enzymes metabolizing MXC *Cyp1a2*, *Cyp2c29* and *Cyp3a4* in the liver of ESR1 OE and control mice. *Cyp1a2* and *Cyp2c29* metabolize MXC, while *Cyp3a4* metabolizes MOH and HPTE to their subsequent metabolites. The data show that at 96h, the cultured antral follicles from ESR1 OE antral follicles are more susceptible to toxicity induced by MXC, MOH and HPTE because low doses of these chemicals caused follicle growth inhibition in ESR1 OE mice, but not in control mice. In the livers, our results showed that while there was no difference in the mRNA levels of *Cyp1a2* and *Cyp2c29* between ESR1 OE and control livers, the mRNA levels of *Cyp3a4* were significantly lower in ESR1 OE livers compared to controls (ESR1 OE fold-change over control = 1: *Cyp1a2* = 1.2; *Cyp2c29* = 1.1; **Cyp3a4* = 0.4; n = 5; *$p \leq 0.05$). Collectively, these data suggest that ESR1 OE mice may be more susceptible to toxicity induced by MXC and its metabolites because of low clearance of the metabolites by the liver. Support: NIH R21ES13061, R01ES012893 and an Eli Lilly Fellowship in Toxicology.

PS 1039 MONO- AND DI-ESTER PHTHALATES ALTER TESTOSTERONE PRODUCTION IN MOUSE BLTK1 MURINE LEYDIG TUMOR CELLS.

Q. Ding[1], N. A. Rahman[2], I. T. Huhtaniemi[2] and T. R. Zacharewski[1]. *[1]Department of Biochemistry & Molecular Biology and Center for Integrative Toxicology, Michigan State University, East Lansing, MI and [2]Department of Physiology, University of Turku, Turku, Finland.*

Phthalates, widely used plasticizers in a variety of consumer and industrial products, have been implicated in the etiology of rodent testicular dysgenesis by altering hormone levels. In this study, BLTK1 cells, a subclone of a murine Leydig cell line isolated from a testicular tumor in a inhibinα/SV40Tag transgenic mouse, are used to study the effects of phthalates and their monoester metabolites (i.e., DMP - mMP, DEP - mEP, DBP - mBP, BBzP - mBzP, DHP - mHP and DEHP - mEHP) on steroidogenesis. BLTK1 cells possess an intact steroidogenic pathway that produces low basal levels of progesterone (P) and testosterone (T) which is inducible by recombinant human chorionic gonadotropin (rhCG) as measured by ELISA. RT-PCR and western blot verified the expression of StAR, Cyp11a, Hsd3b1, Cyp17a1, Hsd17b3 in BLTK1 cells. rhCG (0.01 – 100 ng/ml) exhibited time- and dose-dependent induction of P and T levels with EC50 values of 1.8 and 1.5 ng/ml, respectively, at 4 h. In rhCG time course studies, maximum P induction was achieved at 4 h, which returned to basal levels by 24 h while T levels gradually increased until 8 h and then remained relatively constant. mEHP also elicited marked time- and dose-dependent induction of P and T levels, while DBP, BBzP, and mHP induced moderate levels at 24 h. DMP, mMP, DEP, mEP, mBP, mBzP, DHP, and DEHP elicited negligible effects on P and T levels. QRT-PCR results indicate that mEHP exerts its effects by altering steroidogenic gene expression. More specifically, mEHP induced StAR mRNA levels while repressing the expression of Cyp17a1 and Srd5a1. Overall, these results suggest that selected mono- and di-ester phthalates alter T biosynthesis by regulating the expression of specific steroidogenic genes. Partial funding was provided by the American Chemistry Council.

PS 1040 A STUDY OF FERTILITY IN SPRAGUE-DAWLEY RATS WITH XOMA 052, A NOVEL MONOCLONAL ANTIBODY TARGETING IL-1 BETA.

C. Gasper[1], B. Thorsrud[2], J. Ma[1], L. Cao[1], K. Der[1] and K. Meyer[1]. *[1]Product Development, XOMA (U.S.) LLC, Berkeley, CA and [2]Developmental & Reproductive Toxicology, MPI Research, Mattawan, MI.*

XOMA 052 is an ultra-high affinity (300 fM) humanized IgG2 monoclonal antibody that specifically binds to IL-1 beta and inhibits activation of the IL-1 receptor. This activity is expected to prevent the cellular signaling events that produce inflammation associated with the development of diseases such as Type 2 diabetes, rheumatoid arthritis and Behçet's disease. The rat was selected for this fertility study due to similar binding affinity and *in vitro* functional activity between human and rat IL-1 beta with XOMA 052. Male and female Sprague Dawley rats were administered XOMA 052 or vehicle by subcutaneous injection once weekly at dose levels of 0, 3, 30, and 90 mg/kg. Male rats were dosed weekly starting 28 days prior to pairing and continuing through euthanasia. Female rats were dosed weekly starting 14 days prior to pairing until positive evidence of copulation was observed and again on GD 4. Mated females were each euthanized on GD 13 and all males were euthanized upon competition of the final GD 13 uterine examination. Observations and evaluations included clinical signs, gestation body weights and body weight change, food consumption, immunogenicity, macroscopic pathology, and reproductive performance (estrous cyclicity, reproductive and fertility indices, uterine and ovarian examinations, and sperm evaluation). All animals survived to scheduled necropsy. Results showed no toxicologically significant differences in any of the parameters evaluated. Based on the results, the no-observed-adverse-effect level (NOAEL) for general and reproductive toxicity for male and female rats was considered to be 90 mg/kg, the highest dose level tested. In conclusion, XOMA 052 was well tolerated and did not affect male or female fertility parameters or have any untoward effects during the period of early embryonic development to implantation in the rat.

PS 1041 IMMUNOHISTOCHEMICAL AND CHIP MICROARRAY ANALYSIS OF PPARα IN FETAL RAT TESTES EXPOSED TO DIBUTYLPHTHALATE (DBP).

S. M. Plummer[1], D. Dan[1], J. Quinney[1], M. Millar[3], M. Sheila[3], N. Hallmark[2], R. D. Phillips[2] and C. R. Elcombe[1]. *[1]CXR Biosciences, Dundee, United Kingdom, [2]ExxonMobil Petroleum & Chemical, Hermeslaan, Belgium and [3]MRC Human Reproductive Sciences Unit, Edinburgh, United Kingdom.*

Previous "ChIP on chip" studies indicated an increase in binding of peroxisome proliferator receptor alpha (PPARα) to the Cyp11a promoter in gestational day 15 (GD15) rat testes could account for reduced Cyp 11a expression following DBP exposure (Plummer et al (2010) The *Toxicologist CD*, 114:1488). It was postulated that PPARα hinders transcription factor (SF-1) binding to the Cyp11a promoter, restricting Cyp11a expression and reducing testicular steroidogenesis. To test this hypothesis (1) ChIP microarray data analysis (GD15 and GD19 testes) on additional SF-1-regulated steroidogenic genes and (2) immunohistochemical staining (GD15 testes) for PPARα were conducted. ChIP microarray was performed (see ref. above) on GD15 and 19 fetal testes from Wistar rats exposed *in utero* to DBP (500mg/kg, p.o. to dams from GD12). Bouin's fixed GD15 fetal testes were immunostained using antibodies against PPARα and 3βHSD, a Leydig cell marker. ChIP array data showed DBP increased PPARα binding in StAR and Cyp17a promoter regions in GD15 testes and inhibited SF1-binding in StAR and Cyp17a promoter regions in GD19 testes correlating with reduced expression of these SF-1-regulated steroidogenic genes. Immunostaining results (GD 15) confirmed PPARα protein expression in interstitial regions of the testes partly colocalised with steroidogenic protein 3βHSD in Leydig cells. These data are consistent with expression microarray data (Plummer et al (2007) Toxicol Sci 97:520) and suggest that DBP-induced alterations in PPARα binding could interfere with SF-1 binding in steroidogenic gene promoters. As PPARα is expressed primarily in immature Leydig cells it is likely that interference with SF-1 binding/ transactivation precedes functional maturity in target cells (3βHSD). The data are consistent with the hypothesis that PPARα could in part mediate DBP's anti-androgenic effects. This work was supported by ExxonMobil Petroleum & Chemical

PS 1042 GESTATIONAL EXPOSURE TO ATRAZINE HAS LITTLE EFFECT ON FEMALE REPRODUCTIVE PARAMETERS IN SPRAGUE-DAWLEY (SD) RATS.

L. K. Davis, A. S. Murr, T. E. Stoker, M. Narotsky, J. M. Goldman and R. L. Cooper. *Endocrine Toxicology Branch, NHEERL, U.S. EPA, Research triangle pk, NC.*

Atrazine (ATR), a commonly used herbicide, has been shown to exert reproductive effects in animals; however, most of these studies have focused on adult exposure. In contrast, there is limited information available on the reproductive and developmental effects of gestational exposure to this herbicide. Here, we examined the effects of 0, 1, 5, 20 and 100 mg/kg ATR administered to SD dams on gestational days 14-21, a key developmental window, on female reproductive end points. Dosing occurred once daily or was divided into two doses per day; the lower doses were chosen based on existing female LOELs, while 100 mg/kg served as a positive control. 100 mg/kg decreased both maternal and offspring body weights, and postnatal mortality was higher in this group shortly after birth. By postnatal day (PND) 21 no differences in offspring body weights were present. Age of vaginal opening, used as a pubertal index, was significantly delayed only in the 100 mg/kg group. No differences in body weights were seen at this time. Based on recently established guidelines, mammary gland whole mounts were prepared on PND 45 and analyzed by individuals blind to experimental treatment. There were no apparent treatment effects on mammary gland development, including the number of terminal end buds and branch points. To determine the effects of gestational ATR exposure on estrous cycles in the adult offspring, vaginal smears were examined until the control animals reached reproductive senescence (approximately 9 months). These tracking data indicated that animals in the highest treatment group continued to cycle over this time, while growing numbers of females in the lower dose groups showed an emerging acyclicity. In brief, these data indicate that gestational exposure to ATR at doses as high as 100 mg/kg in the dam, had few adverse effects on these standard measures of reproductive development. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

PS 1043 THE EFFECTS OF ENDOCRINE DISRUPTION ON THE DEVELOPING HUMAN FETAL PROSTATE.

C. Saffarini, S. J. Hall and K. Boekelheide. *Brown University, Providence, RI.*

Prostate cancer is the most commonly diagnosed cancer and is the second highest cancer-related cause of death in men. This high incidence has led to speculation about the fetal origins of this adult disease and its etiology. Exposure to exogenous estrogens in pregnant women has been postulated to disturb the normal development of the human fetal prostate by disrupting the natural hormonal balance. Previous studies demonstrate that exposure to estrogens can cause some degree of epithelial and stromal hyperplasia, inflammation, and prostatic intraepithelial neoplasia (PIN) lesions. The present study used a xenograft model to characterize and determine the differentiation of the human fetal prostate implants (gestation 12-22 weeks) exposed to either corn oil (control) or 250 ug/kg/body weight of 17β-estradiol 3-benzoate during the first few days post-transplant. This xenograft model uses the renal subcapsular space as the site of implantation, thereby allowing for proper vascularization and growth of the implant. Immunohistochemical (IHC) characterization included markers specific for stromal (desmin, smooth muscle alpha actin, myosin, vinculin, vimentin) and epithelial maturation (cytokeratins, lamin A/C, e-cadherin, p63, prostate specific antigen), neuroendocrine cells (chromogranin A), and hormone receptors (androgen receptors, estrogen receptor alpha and beta). In addition, proliferation as well as apoptosis was noted using KI-67 and TUNEL staining. As expected, the prostate implants appear to grow and mature as seen in the 7, 14 and 30 day time points following xenografting. Interestingly, preliminary IHC results reveal a decrease in both KI-67 and e-cadherin expression, as well as an increase in PSA expression in the 7-day estradiol treated implants. This unique xenograft model will provide insight on the growth and development of the human fetal prostate following developmental endocrine disruption.

PS 1044 TWO-GENERATION REPRODUCTIVE TOXICITY STUDY OF ALUMINIUM SULFATE ADMINISTERED *VIA* DRINKING WATER TO RATS.

M. Hirata-Koizumi[1], S. Fujii[2], A. Ono[1], A. Hirose[1], R. Hasegawa[1], T. Imai[3], K. Ogawa[1], M. Ema[1] and A. Nishikawa[1]. *[1]National Institute of Health Sciences, Tokyo, Japan, [2]Safety Research Institute for Chemical Compounds Co., Ltd., Sapporo, Japan and [3]Central Animal Laboratory, National Cancer Center Research Institute, Tokyo, Japan.*

In order to evaluate the human health risk by intake of aluminium from food and drinking water, the 67th JECFA meeting clearly stated the need for a multigenerational study on a relevant aluminium compound. We conducted a two-generation reproductive toxicity study (OECD GD416) in SD rats given drinking water containing aluminium sulfate at 0, 120, 600 or 3000 ppm. In both F0 and F1 generations, water consumption was decreased in males and females of all treatment groups. Decreased food consumption was observed in both sexes of F0 and F1 animals in the 600 and 3000 ppm groups, and the body weight in F0 males and females was decreased only at 3000 ppm. In either generation, no compound-related changes were found in the reproductive parameters. While there were also no changes in birth weight of F1 and F2 pups, decreased body weights on postnatal day 21 were observed in male and female F1 pups and female F2 pups in the 3000 ppm group. Although there were decreases in the liver and spleen weight in both sexes of F1 and F2 weanlings at 3000 ppm, no histopathological changes were detected in these organs. Vaginal opening was slightly delayed in F1 females at 3000 ppm, but there was no significant difference in the body weight at the time of attainment between the control and 3000 ppm groups. There were no compound-related changes in other developmental parameters, including behavioral parameters. In conclusion, the NOAEL for the reproductive/developmental toxicity was considered to be 600 ppm, which was corresponding to the total aluminum intake of 8.06 mg Al/kg bw/day in rats by considering aluminium content in the standard diet (25-29 ppm).

PS 1045 APPLICATION OF *IN VIVO* AND *IN VITRO* TOOLS FOR DETECTION OF EMBRYOTOXICITY: THE EXAMPLE OF ARTEMISININ-TYPE COMPOUNDS.

P. Colombo, M. Longo, S. Zanoncelli and M. Brughera. *Preclinical Development, Accelera Srl, Nerviano - Milano, Italy.*

The investigation of embryotoxicity using conventional in vivo experimental designs (e.g. ICH S5A), can be enhanced when combined with a panel of complementary in vitro embryotoxicity tests, namely the rat whole embryo culture, micromass, frog embryo and zebrafish embryo teratogenesis assays. Here we present a case in which a combination of in vivo and in vitro embryotoxicity assays were applied to the study of the anti-malarial drug dihydroartemisinin, demonstrating the potential use of in vitro data during drug discovery as an early stage indicator of potential liabilities. Artemisinin derivatives are a cornerstone of contemporary malaria treatment, although embryotoxicity remains a critical issue, as infection with Plasmodium falciparum is dangerous to both mother and child during the first trimester of pregnancy. Initial conventional in vivo studies indicated that artemisinin-type compounds were embryolethal in experimental animals through an unknown mechanism. Subsequent ad hoc in vivo studies in the rat helped identify a narrow window of susceptibility of the embryo to artemisinins. Complimentary in vitro assays were then able to identify a species-nonspecific mechanism of embryotoxicity: the depletion of primitive metabolically active RBCs (Red Blood Cells) clonally released over a short period of time from primitive sites of haematopoiesis. In vitro assays further helped in establishing differences in the length of the period of susceptibility vs. the period of exposure (treatment duration) helping the evaluation of the clinical relevance of this finding. Data obtained using in vitro models can also provide useful feedback to drug discovery, allowing ranking of novel potential compounds according to embryotoxicity liability. In an attempt to establish whether and to what extent activity as anti-malarials and embryotoxicity can be divorced, IC50s for activity in P. falciparum strains and the NOELs for RBCs were compared for various compounds, demonstrating that in certain cases a good safety margin can exist.

PS 1046 DEVELOPMENT OF REPRODUCTIVE ORGANS IN MALE CYNOMOLGUS MONKEYS.

E. Haruyama[1, 2], Y. Ayukawa[1], K. Kamura[1], M. Mizutamari[1], Y. Ooshima[1], T. Sukamoto[1], R. Nagata[1] and A. Tanimoto[2]. *[1]Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Kagoshima, Japan and [2]Kagoshima University, Kagoshima, Kagoshima, Japan.*

Purpose: We examined reproductive organs in control group cynomolgus monkeys (136 males) used in toxicity studies from August 2008 to August 2009 to evaluate their development histopathologically and morphometrically. Result: A clear correlation was noted between histopathological degree of testicular maturity, as classified into six grades (1: Immature, 2: Pre-pubertal, 3: Onset of puberty, 4: Pubertal, 5: Early adult, 6: Adult) and testis weight; however, large individual differences in the relationships with age and body weight were recognized. Increases in the epididymis and seminal vesicle weights accompanying increased testis weight were indicated. The prostate showed an increase in weight accompanying increased testis weight; however, there was a tendency to remain constant at Grades 4 to 6. When compared by the degree of testicular maturity in morphological examinations, the areas of the ductus epididymis and the cavity of the ductus epididymis showed increases with grade, and were notably increased at Grade 6. The proportion of the epithelial cell layer of the seminal vesicles also showed an increase with grade. In the prostate, glandular cavities were generally not observed with Grade 1; however, they were visible from Grade 2, and increases in relative glandular cavity area were noted up to Grade 4. Conclusion: In these results, a clear correlation between testis weight and histopathological degree of maturity was noted; however, there were large individual differences in the relationships with age and body weight, and it is considered necessary to select animals for toxicity studies in which testicular toxicity it to be evaluated. The prostate does develop in a manner accompanying the stage of testicular development; however, maturity is reached at Grade 4. Conversely, the epididymides and seminal vesicles develop in accordance with the degree of testicular maturity, and differences in development applying to each organ were noted.

PS 1047 INTERACTIONS OF ENDOCRINE DISRUPTING CHEMICALS WITH THE BCRP TRANSPORTER: POTENTIAL MECHANISM FOR DICTATING FETAL EXPOSURE.

L. Aleksunes and X. Wen. *Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Efflux transporters are critical determinants of environmental chemical disposition and susceptibility to adverse events. Because in utero exposure to chemicals that interfere with endocrine function carries the risk of developmental perturbations, it is important to understand how endocrine disrupting chemicals (such as bisphenol A, genistein, zearalenone, methoxychlor) interact with placental efflux transporters such as the breast cancer resistance protein (BCRP), multidrug resistance protein 1 (MDR1), and multidrug resistance-associated proteins 1, 2, and 5 (MRP1, 2, 5). The purpose of this study was to identify interactions between efflux transporters and endocrine disrupting chemicals using membrane vesicles overexpressing human isoforms of MRP1, 2, 5, MDR1, and BCRP. Functional inhibition studies of endocrine disrupting chemicals were conducted using fluorescent prototypical substrates and inside-out membrane vesicles overexpressing human BCRP, MDR1, MRP1, 2, and 5. All four endocrine disrupting chemicals markedly inhibited BCRP transport (10-40% of control) while only bisphenol A reduced MDR1

transport (72% of control). MRP2 transport was only inhibited by genistein (75% of control). None of the chemicals tested altered MRP5-mediated transport. Additional studies demonstrated that metabolites of methoxychlor (including HPTE and mono OH-methoxychlor) inhibited BCRP transport in membrane vesicles. Preliminary cytotoxicity studies suggested that BCRP mediates direct transport of genistein and zearalonone in BCRP-expressing HEK293 cells as evidenced by increased cytotoxicity of both chemicals in the presence of the BCRP inhibitor, Ko143. Inhibition of BCRP function in the placenta by endocrine disrupting chemicals may increase exposure of the fetus to environmental contaminants and pharmaceuticals and enhance susceptibility to developmental adverse events. Ongoing studies are investigating the ability of BCRP in placenta cells to transport endocrine disrupting chemicals at environmentally-relevant concentrations (Supported by ES-005022).

PS 1048 THE EFFECTS OF TRIS (2-ETHYLHEXYL) TRIMELLITATE (TOTM) ON GENE EXPRESSION ASSOCIATED WITH TESTICULAR MAL-DEVELOPMENT (TMD) IN RAT FETAL TESTES.

C. R. Elcombe[1], D. Dan[1], D. G. Farrar[2] and S. M. Plummer[1]. *[1]CXR Biosciences, Dundee, United Kingdom and [2]INEOS Chlor Limited, Runcorn, United Kingdom.*

To assess the potential of tris (2-ethylhexyl) trimellitate (TOTM) to induce testicular mal-development (TMD) in the rat we studied its effects on expression of genes in steroidogenesis/testes development pathways, thought to be involved in TMD induction by certain phthalates. The effects of TOTM were compared with those of di(2-ethylhexyl phthalate (DEHP), mono-(2-ethylhexyl) phthalate (MEHP), an active metabolite of DEHP, and with 2-ethyl hexanol (EHO), considered to be an 'inactive' DEHP metabolite. Wistar rats were exposed to TOTM, MEHP, DEHP, EHO (500 mg/Kg) or corn oil (vehicle) *in utero* by oral gavage to pregnant dams on gestational days (GD) 12-19. A low-dose DEHP (0.25mg/Kg) treatment group was also included as DEHP can be a minor contaminant of the technical grade of TOTM. Whole Rat Genome Microarrays were used to screen for gene expression changes in TMD pathways using RNA isolated from GD19 fetal testes. MEHP & DEHP (500 mg/Kg) caused a repression of genes in TMD pathways involved in cholesterol synthesis and transport (HMGCS, HMGCR, StAR, SCARB1, FDFT1, FDPS), steroidogenesis (Cyp11a, Cyp17a, 3βHSD1, SC4MOL) and testes positioning/function (INSL3, INHA). EHO, induced weak repression StAR, HMGCS, INHA and SCARB1, but did not repress Cyp 11a, Cyp17a, 3βHSD1, SC4MOL or INSL3. By contrast TOTM or DEHP (0.25mg/Kg) did not cause a significant repression of TMD-pathway genes. We conclude that TOTM, and its technical grade, would be unlikely to cause testicular dysgenesis in foetal rats under these treatment conditions. The effects of EHO can be partly rationalised on the basis of it being a weak PPARα agonist. Our recent work on mechanisms of phthalate-induced TMD suggests that the ability of certain phthalates to repress steroidogenic genes, including StAR, is mediated by direct binding of PPARα to the promoter regions of steroidogenic genes (Plummer et al (2010) The Toxicologist CD, 114:1488). This work was supported by INEOS Enterprises Limited, Polynt SpA, Oxea Deutschland GmBh and Novasol N.V/S.A.

PS 1049 EPIVAGINAL TISSUE MODEL FOR PRECLINICAL SCREENING OF SINGLE OR REPEAT EXPOSURE TO VAGINALLY APPLIED CHEMICALS/FORMULATIONS.

C. Cannon, M. Klausner, S. Ayehunie, A. Wang, K. LaRosa and T. Landry. *MatTek Corp, Ashland, MA.* Sponsor: P. J.

A predictive test system for assessing the vaginal irritation potential of chemicals and formulations will have far reaching application in the testing of feminine care products. The vaginal mucosa is commonly exposed to chemicals and therapeutic agents that may result in irritation/inflammation which can make women susceptible to infections such as HIV-1 and HSV-2. Hence, chemical or therapeutic agent induced vaginal irritation is a public health concern. In the current study, we investigated whether the highly differentiated EpiVaginal™ tissue of normal human vaginal/ectocervical cells could be used as a non-animal alternative for the FDA approved rabbit vaginal irritation (RVI) assay. The tissue was exposed to 1X (24 hr) or 5X (1 hr/day for 5 days) to 6 test articles (TA). Tissue viability (MTT assay), barrier disruption (measured by trans-epithelial electrical resistance, TEER and sodium fluorescein (FL) leakage), and inflammatory cytokine release (IL-1α, IL-1β, IL-6, and IL-8) were examined. Two irritating TA, benzalkonium chloride (BC) and nonoxynol 9 (N9), reduced tissue viability to <40% and TEER to <60 % and increased FL leakage by 11-24% and IL-1α and IL-1β release by >100% in the 1X test. 4 other non-irritating TA and vaginal fluid simulant (pH = 4.2) had minimal effects on these parameters. Assay reproducibility was confirmed by testing using 3 different production lots (<10%CV) and by using tissues derived from cells from 3 different donors (<12% CV). In the repeat application experiments, BC and N9 reduced tissue viability to <10% and TEER <20% IL-1α and IL-1β release after 24 hours were found to be indicators of chemical toxicity. In conclusion, decreases in MTT and TEER and increases in FL leakage and IL-1α and IL-1β release are useful endpoints for preclinical toxicity screening of chemicals/formulations. The EpiVaginal method will be rapid, easy to use, economical, and reproducible, and it should be further evaluated as a potential alternative to the RVI assay.

PS 1050 IVOS AND HISTOPATHOLOGY PROCEDURES FOR GLP-COMPLIANT TOXICOLOGY STUDIES IN NONHUMAN PRIMATES.

R. E. Watson[1], D. Thompson[1], M. Novilla[1], N. Makori[1], R. Eyre[1] and R. Nagata[2]. *[1]SNBL USA, Seattle, WA and [2]SNBL Laboratories, Kagoshima, Japan.*

Assessment of male fertility parameters is key to preclinical drug development. This study aims to optimize GLP-compliant male fertility analysis in the cynomolgus monkey. Semen parameters, male reproductive organ weights and histopathological findings in 4 monkeys implanted with testosterone (T) capsules for 20 wks vs. 4 monkeys with saline capsules were measured. This T treatment regimen had previously been shown to reduce semen concentration and testicular volume without causing signs of overt toxicity. Using the Integrated Visual Optical System (IVOS) system, semen concentration and motility were measured during wks 8, 16, and 20 using a standard validated method that assesses these parameters separately, and a proposed method assessing these parameters simultaneously. At necropsy on wk 20, a histopathology approach was used to identify cell and spermatogenic stage related effects. Slides of male reproductive tissues were stained with hematoxylin and eosin; testis sections were also stained with periodic acid Schiff reaction. Additional testis sections were stained with periodic acid Schiff and a hematoxylin counterstain. Treatment-related effects on semen concentration, organ weight, and histopathological findings were seen. Validated and proposed IVOS methods demonstrated similar percent decreases in sperm concentration from acclimation to wk 20 in treated animals. Reductions of 37.6, 19.4, 90.0, and 76.4% were seen in each animal using the validated method; corresponding reductions with the proposed method were 36.1, 24.8, 94.4 and 92.9%. Mean absolute weights of the testis, epididymis, prostate and seminal vesicles in treated animals decreased by 58.4, 35.9, 35.4, and 16.5% compared to controls. Loss of testicular germ cells, atrophy of the seminiferous tubule epithelium, reduced Leydig cell size, and reduced epididymis sperm content were seen in treated animals. In conclusion, the proposed IVOS method and histopathological approach have shown promise in efficiently assessing male fertility parameters in a GLP toxicology lab setting.

PS 1051 GOLD NANOPARTICLE EFFECTS ON RAT OVARIAN GENE EXPRESSION.

J. Larson[1, 3], M. J. Carvan III[2, 3], R. Klaper[2, 3] and R. J. Hutz[1, 3]. *[1]University of Wisconsin Milwaukee, Milwaukee, WI, [2]University of Wisconsin Madison Great Lakes WATER Institute, Milwaukee, WI and [3]NIEHS Children's Environmental Health Sciences Core Center, University of Wisconsin Madison and Medical College of Wisconsin, Milwaukee, WI.*

Gold nanoparticles (GNP) have gained attention for use in drug-delivery, bio-imaging, and cancer therapies. However, research exploring potential harmful effects of GNP on female fertility is almost nonexistent. We have demonstrated that 10-nm GNP enter rat ovarian granulosa cells, alter mitochondrial morphology, and significantly reduce estradiol-17β (E_2) accumulation after 24 hours of incubation. Moreover, we have shown using a rat ovarian culture model that 10-nm GNP modulates progesterone (P_4) accumulation in a dose- and time-dependent manner, but does not alter E_2 accumulation. The objective of the present study was to evaluate the locus (loci) of modulation by 10-nm GNP (and any time- and dose-dependent effects) on ovarian steroidogenesis *in vitro* and exploit multiple-reference gene quantitative real-time RT-PCR (QPCR) to evaluate expression of key steroidogenic and oxidative stress-related genes. We hypothesized that GNP modulate ovarian steroidogenesis by affecting expression of one or more genes involved in sex-steroid biosynthesis (*i.e.*, *cyp11a1* and *cyp17a11*) and/or oxidative stress (*i.e.*, *sod2* and *hmox1*). Ovaries were cultured under control or treatment conditions (2.85×10^4, 2.85×10^7, or 2.85×10^{10} particles/mL medium) for three different time periods (12, 24, or 48 hours). Individual one-way ANOVAs revealed no significant differences in the expression of *cyp11a1*, *cyp17a1*, *sod2*, and *hmox1*, relative to control, as a function of GNP concentration after 12 or 48 hours *in vitro* ($p>0.05$). Individual t-tests also showed no significant differences in the expression of *cyp11a1*($p=0.801$), *cyp17a1*($p=0.103$), *sod2*($p=0.068$), or *hmox1*($p=0.087$) after 12 or 48 hours incubation . These results suggest that 10-nm GNP modulate P_4 accumulation in our *in-vitro* paradigm via a mechanism that may be independent of oxidative stress and transcriptional regulation of *cyp11a1* and *cyp17a1*. Research funding was provided by the CEHSCC at UWM and MCW.

PS 1052 MONO-2-ETHYLHEXYL PHTHALATE INCREASES OXIDANT SPECIES PRODUCTION AND MODIFIES EXPRESSION OF REDOX-SENSITIVE GENES IN HUMAN PLACENTAL CELLS.

L. M. Tetz and R. Loch-Caruso. *Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI.*

Di-2-ethylhexyl phthalate (DEHP) is a ubiquitous environmental pollutant used mainly as a plasticizer in polyvinyl chloride (PVC) products. Mono-ethylhexyl phthalate (MEHP), the active metabolite of DEHP, increases reactive oxygen species production and decreases antioxidant levels in liver, kidney, and testicular cells. To investigate whether the placenta is a potential target of MEHP, we evaluated the effects of MEHP on production of reactive oxygen species and expression of redox-sensitive genes in immortalized human placental (extravillous trophoblast) cells (HTR-8/SVneo). Stimulation of oxidant species generation was assessed by formation of the fluorescent product 2',7'-dichlorofluorescein (DCF) in HTR-8/SVneo cells loaded with 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate and exposed for 1 h to dimethyl sulfoxide (DMSO; solvent control), or 12.5, 25, 50, 100 or 200 μM MEHP. MEHP (100 and 200 μM) significantly increased oxidant species production 90 min after cessation of exposure. RNA expression of 84 redox sensitive genes was measured in cells exposed to 100 μM MEHP for 4, 8 or 24 hours (n=3 experiments) using a RT-PCR array. Six genes were significantly changed two-fold or more in cells exposed to MEHP compared to solvent control. These included oxidative stress-responsive genes (NME5, NOX5, and TXNRD2), genes important during pregnancy (PTGS2), and genes potentially involved in placental development (TTN and ANGPTL7). This study shows that MEHP increases oxidant production and modifies expression of redox-sensitive genes, most notably, PTGS2, a gene that is necessary for initiation of parturition.

PS 1053 RELATIONSHIP BETWEEN LOW METABOLIC CAPABILITY OF LIGNANS AND IDIOPATHIC MALE INFERTILITY.

Y. Xia, P. Zhu, M. Chen, C. Lu, S. Wang and X. Wang. *Institute of Toxicology, Nanjing Medical University, Nanjing, China.*

Aim: To investigate the relationships between urinary levels and metabolic capability of phytoestrogens (PEs) and idiopathic male infertility in Chinese adult males. Methods: Through eligibility screening procedures, 688 idiopathic infertile men and 469 fertile controls were recruited. Based on their semen volume, sperm concentration, sperm number per ejaculum and sperm motility, subjects with idiopathic infertility were further divided into "normal" and "abnormal" semen quality groups. By using UPLC-MS/MS, individual exposures to PEs were evaluated by urinary concentrations of eight PEs or their metabolites (secoisolariciresinol, SEC; enterolactone, ENL; enterodiol, END; daidzein, DAI; genistein, GEN; equol; naringin; coumestrol), which were adjusted by creatinine. Results: The median adjusted isoflavone and lignan concentrations were higher or lower than those in the U.S reports, respectively. The adjusted SEC, GEN and DAI concentrations of infertile group were significantly higher than those of control group. Subjects with higher SEC, GEN and DAI levels were more likely involved in idiopathic male infertility with abnormal semen quality ($P<0.01$, 0.05 and 0.05, respectively). However, subjects with higher END and ENL (metabolites of SEC) levels were less possibility to have idiopathic male infertility ($P<0.05$, and 0.01, respectively). Furthermore, the percentage of the men who can not metabolism SEC into END/ENL in infertile group were much high than those in control group ($P<0.01$). Dysmetabolic rate of SEC, represented as SEC/(SEC+END+ENL), also showed the significant association with idiopathic male infertility ($P<0.01$). Conclusion: High level urinary concentrations of SEC, GEN and DAI were found associated with increased risk of male idiopathic infertility. Furthermore, the idiopathic infertile subjects with abnormal semen might be in higher risk. Low metabolic capability of SEC significantly associated with idiopathic male infertility and abnormal semen quality. These findings should be of concern because of the ubiquitous exposure of PEs.

PS 1054 INHIBITION OF OVARIAN KIT AUTOPHOSPHORLATION BY THE OVOTOXICANT 4-VINYLCYCLOHEXENE DIEPOXIDE.

C. J. Kappeler[1], L. McKee[1], I. Sipes[2], J. Konhilas[1] and P. B. Hoyer[1]. [1]*Physiology, University of Arizona, Tucson, AZ and* [2]*Pharmacology, University of Arizona, Tucson, AZ.*

In vitro exposure of Postnatal Day 4 (PND4) rat ovaries to the occupational chemical 4-vinylcyclohexene diepoxide (VCD) specifically destroys ovarian primordial and primary follicles via an increase in the natural process of atresia. The interaction of granulosa cell-derived kit ligand (KITL) with oocyte cell-derived c-kit (KIT) plays a critical role in primordial follicle activation and oocyte survival and growth. KITL binding to the tyrosine kinase receptor KIT results in dimerization and autophosphorylation of KIT, and ultimately the activation of multiple signaling pathways. Earlier studies found that, following exposure to VCD there is a decrease in Kit mRNA (d4) and an increase in Kitl mRNA (d6). Further, VCD decreased activities of downstream members of the KIT/KITL signaling pathway as early as d2 of VCD exposure. Thus, these findings supported that the earliest observed event of VCD-induced ovotoxicity is via disruption of the KIT signaling pathway. Therefore this study was designed to investigate a possible direct interaction of VCD with KIT as its mechanism of ovotoxicity in primordial and small primary follicles. PND4 Fischer 344 rat ovaries were cultured in control media ± VCD (30 μM) for 2d. Phosphorylated KIT (pKIT) and total KIT protein were assessed by Western analysis. pKIT protein was decreased ($P<0.05$) within 2d of VCD exposure (48% decrease), while total KIT protein was not affected by VCD at this time. In addition, mobility shift detection of phosphorylation of KIT was performed using a Mn2+-Phos-tag SDS-PAGE gel followed by Western analysis. By this method, there was also a decrease ($P<0.05$) in pKIT protein compared to total KIT protein. These findings demonstrate that VCD induces ovotoxicity by a direct inhibition of KIT autophosphorylation on the oocyte, thereby resulting in a reduction of KIT signaling and its role in primordial follicle activation and survival. (T32-ES007091, R01-ES09246, and Center grant ES06694)

PS 1055 A DOSE-RESPONSE STUDY FOLLOWING LATE GESTATIONAL EXPOSURE TO THE PHTHALATE METABOLITE, MONO-2-ETHYLHEXYL PHTHALATE (MEHP): EFFECTS ON FEMALE MOUSE REPRODUCTIVE DEVELOPMENT.

B. J. Moyer and M. L. Hixon. *Brown University, Providence, RI.*

Premature ovarian depletion may have causes associated with environmental exposures, but the extent and mechanisms of these possible associations are as yet poorly understood. Our current study demonstrates that exposure to high doses of mono-2-ethylhexyl phthalate (MEHP) in late gestation results in premature ovarian failure in the adult female mouse. Pregnant C57/BL6 mice were exposed via oral gavage to corn oil, 100, 500, or 1000 mg/kg MEHP at gestational days 17 through 19. No overt maternal toxicity was observed. No significant delays in vaginal opening were observed and body and ovarian weights were similar between vehicle- and MEHP-exposed adult females. Females exposed in utero exhibited a delay in estrus at both the 500mg/kg and 1000mg/kg MEHP exposure groups. A prolonged estrus stage and decreased instance of diestrus stage in the estrous cycle was observed at all dosage groups. Serum FSH levels at estrus were significantly higher in the 1000mg/kg in utero exposure group. In addition, increased mRNA expression levels were observed for LHCGR and StAR. Collectively, these findings suggest that late gestational exposure to phthalates leads to altered ovarian function and steroidogenesis in adult female mice.

PS 1056 TETRABROMOBISPHENOL A STIMULATES SECRETION OF INTERLEUKIN 6 FROM HUMAN FIRST TRIMESTER TROPHOBLASTS.

P. W. Kimani and R. Loch-Caruso. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Background: Extravillous trophoblast invasion of maternal uterine tissue during early pregnancy is integral to facilitate exchange of nutrients between the maternal and fetal circulatory systems. These trophoblasts secrete inflammatory cytokines and chemokines that recruit immune cells to the maternal-fetal interface, to aid in remodeling of the maternal spiral arteries. Failure of this process has been associated with a number of pathologies including intrauterine growth restriction and preeclampsia. Effects of exposure to tetrabromobisphenol A (TBBPA) on this process have not been studied, yet TBBPA is one of the most widely used flame retardants in the world, is an environmental pollutant and has been detected in human gestational membranes. The aim of our study was to determine whether TBBPA could stimulate cytokine and chemokine secretion from trophoblasts. Method: We exposed HTR8/SVneo human first trimester extravillous trophoblast cells to TBBPA for 4, 16 or 24 h. We then used enzyme-linked immunosorbent assay (ELISA) to measure the levels of IL-6 and IL-8 in cell culture medium obtained from our four treatment groups: non-treated control (NT), solvent control (dimethyl sulfoxide, DMS0), 5 uM TBBPA and 10 uM TBBPA. We selected the TBBPA concentrations based on initial cytotoxicity studies. Results: We detected significantly higher levels of IL-6 at all time points compared to the controls ($p<0.001$) and the 5 uM TBBPA group ($p<0.001$). In contrast, there were no treatment-related differences in IL-8 concentrations. Conclusions: Our results demonstrate that TBBPA stimulated IL-6 secretion in human trophoblasts. To our knowledge, this is the first study examining TBBPA-mediated toxicity in human placental

cells. Given the importance of trophoblast-derived cytokines in pregnancy, further research is warranted to ascertain potential relevance of these findings to human health.

PS 1057 OXIDANT-INDUCED INFLAMMATORY RESPONSE IN HUMAN PLACENTAL CELLS IS DEPENDENT ON MITOGEN ACTIVATED PROTEIN KINASES (MAPKS).

C. S. Korte and R. Loch-Caruso. *Environmental Health Science, University of Michigan, Ann Arbor, MI.*

Preterm birth is associated with significant infant morbidity and mortality. Although the etiology of preterm birth is not fully understood, it is well-established that labor is induced by inflammatory activity within the gestational compartment. Labor also is associated with markers of oxidative stress within gestational tissues, but the impact of oxidative stress on labor-promoting pathways has received little attention. The present study examined the effect of oxidative insult, in the form of tert-butyl hydroperoxide (TBHP) exposure, on MAPK-dependent inflammatory cytokine release from placental cells using the human extravillous trophoblast cell line HTR-8/SVneo. HTR-8/SVneo cells were treated with 12.5, 25 or 50μM TBHP for 2, 6 or 24 h in the presence or absence of pharmacological MAPK inhibitors. Cytokine release was then measured by specific ELISA. At 24 h, 50 μM TBHP induced a significant 2.5-fold increase in interleukin (IL)-6 release into the culture medium, but IL-8 release was not significantly affected. Inhibition of p38 MAPK and ERK1/2 but not JNK decreased TBHP-induced IL-6 release. In contrast, exposure to the endotoxin lipopolysaccharide (LPS) stimulated release of both IL-6 and IL-8, but release of IL-6 and IL-8 was dependent on p38 MAPK alone. Further research is needed to understand the implications of the differential MAPK pathway activation and cytokine release elicited by TBHP compared with LPS. Nonetheless, these data indicate that oxidative insult induces inflammatory cytokine release from trophoblast cells by specific MAPK pathways. Because elevated amniotic fluid concentrations of IL-6 are strongly associated with preterm birth in women, toxicant exposures that induce oxidative stress within the gestational compartment may pose a risk for pregnancy.

PS 1058 REGULATION OF HUMAN PLACENTAL DRUG TRANSPORTERS IN RESPONSE TO BACTERIAL CELL WALL COMPONENTS.

L. Yacovino[1], A. Vetrano[2], N. Hanna[3] and L. M. Aleksunes[1]. [1]*Pharmacology & Toxicology, Rutgers University, Piscataway, NJ,* [2]*Pediatrics, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ and* [3]*Pediatrics, Winthrop University Hospital, Mineola, NY.*

Efflux drug transporters within the placenta are key defense mechanisms for limiting fetal exposure to xenobiotics. Placental drug efflux transporters include isoforms of the Multidrug Resistance-Associated Protein (MRP) family as well as the Multidrug Resistance Protein 1 (MDR1) and the Breast Cancer Resistance Protein (BCRP). Two chemicals of particular interest are lipoteichoic acid (LTA) and lipopolysaccharide (LPS), both components of bacteria able to initiate an inflammatory response and potentially regulate drug transporter expression within the placenta. The purpose of this study was to quantify changes in transporter mRNA expression in term human placentas 24 h after ex vivo LPS and LTA exposure. Preliminary mRNA analysis demonstrated a 2-fold increase in TNF-alpha cytokine mRNA as well as elevated levels of MRP2 mRNA in LPS- and LTA-exposed placentas. Expression of BCRP and MRP1 mRNA was variable among placentas and unchanged by LPS and LTA treatment. MDR1 and IL-6 mRNA levels were below the level of detection. Additional studies are underway to quantify time-dependent changes in transporter mRNA and protein expression in response to LPS and LTA stimulation in additional human placentas. In addition, transcriptional and epigenetic mechanisms by which changes occur in mRNA and protein expression will be investigated. Altered expression of placenta drug transporters during periods of infection and inflammation may alter the maternal-fetal disposition of endogenous chemicals and toxins (Supported by ES-007148 & ES-005022).

PS 1059 ALCOHOL DRINKING-PROMOTED ACETALDEHYDE ACCUMULATION AND OXIDATIVE STRESS IN RAT TESTES.

G. D. Castro, L. N. Quintans, F. M. Bietto, M. I. Díaz Gómez and J. A. Castro. *Ceitox, Conicet-Unsam, San Martín, Buenos Aires, Argentina.*

Excessive alcohol consumption is associated with impaired testosterone production and testicular atrophy. Similar findings were observed in in vitro studies on the testosterone production by isolated testes, being acetaldehyde (AC) more potent than alcohol in suppressing testosterone release. In previous studies we reported that rat testicular microsomes were able to bioactivate ethanol to AC, 1-hydroxyethyl and hydroxyl radicals. Now we report that after a single dose of ethanol (3.8 gr/kg, ig.), AC accumulates in testicular tissue for prolonged periods of time (about 9 hours). Comparison against alcohol or AC concentrations in blood and liver tissue up to 24 hours suggests that AC generation in situ is determinant for this cumulative effect. We also observed that testicular cytosolic ADh, xanthine oxidase and AldDh activities were very low or undetectable by histochemical procedures. Six hours after that single dose of ethanol the testicular levels of lipids hydroperoxides (xylenol orange method) were significantly increased. Similar observations were made in testes from animals receiving a standard Lieber and De Carli diet during 28 days. We also tested twenty four polyphenols having known antioxidant properties against the NADPH dependent and the NADPH non-dependent microsomal metabolism of alcohol to AC. Some of them exhibit significant inhibitory effects at low concentrations (10-50 micromolar) on both metabolic processes. Results suggest that in situ microsomal metabolism of alcohol to AC and the low AldDh activity present at the testicular tissue might be involved in the AC accumulation observed, and that our previously reported alcohol-promoted generation of 1-hydroxyethyl and hydroxyl radicals might initiate the lipid peroxidation process leading to production of the lipid hydroperoxides detected in vivo. Ability of polyphenols to inhibit these microsomal pathways of alcohol metabolism at low concentrations suggest preventive effects of them against alcohol induced testicular damage and reproductive impairment, that remain to be established.

PS 1060 NONHUMAN PRIMATE POSTNATAL DEVELOPMENT: HISTORICAL CONTROL DATA IN NONCLINICAL TOXICOLOGY STUDIES.

N. Lalayeva[1], J. Kenfield[1], J. Cowan[2], S. Oneda[1], N. Makori[1], R. Eyre[1], H. Tsusaki[1] and R. Nagata[3]. [1]*SNBL USA, Ltd., Everett, WA,* [2]*Consultant for NHP Infant Behavior, San Francisco, CA and* [3]*Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan.*

Extended functional and morphological evaluations of infant nonhuman primates are essential components of pre-/post natal developmental toxicology studies based on addendum to ICH 6S (R1) guideline. The aim of this study is to present comprehensive historical control background data collected in infants up to seven months post delivery. Infants were evaluated using the following tests under GLP conditions: incidence of mortality at natural delivery, external teratological examination at birth, skeletal development (post-natal day [PND] 30), external morphometrics (PNDs 3, 30, and 95), behavioral assessment (PNDs 45, 105, and 195), body weights (weekly), and learning and memory using a Wisconsin General Testing Apparatus (WGTA). Incidence of stillbirths by study ranged from 0% to 17.6%, with no correlation to gestation day of birth. Average infant body weights (genders combined) at birth were 323.3 g (220.4 g to 441.8 g) and 404.4 g (276.0 g to 510.4 g) one month after birth. No abnormalities were observed during external morphological or skeletal examinations. Similarities in behavioral characteristics were demonstrated in >90% of infants. WGTA testing showed the number of sessions to criterion for the training phase to be 5 (range 2 to 11), 13 (range 4 to 26) for the learning phase, and 6 (range 3 to 12) for the 9 out of 17 animals that completed the first reversal. The number of reversals completed within the 30-session series was on average 2 (range 0 to 7). In conclusion, robust control background data is a useful reference tool for results interpretation in pre-/postnatal reproductive toxicology studies.

PS 1061 MENSTRUAL CYCLE AND SERUM HORMONE DATA IN MATURE CYNOMOLGUS MONKEYS.

S. Oneda[1], N. Lalayeva[1], R. Watoson[1], N. Makori[1], R. Eyre[1], H. Tsusaki[1] and R. Nagata[2]. [1]*SNBL USA, Ltd., Everett, WA and* [2]*Shin Nippon Biomedical Laboratories, Ltd., Tokyo, Japan.*

Introduction: The cynomolgus monkey (*Macaca fascicularis*, Mfl) is a useful animal model for nonclinical developmental and reproductive toxicity studies of biopharmaceuticals. Female fertility can be assessed in a repeated dose toxicity study using sexually mature monkeys [ICH Guideline S6 (R1)], and menstrual cyclicity is one of the critical parameters. The goal of this presentation is to establish normal range of menstrual cycle (MC) length and criteria of abnormal cycles in Mfl. Serum 17-beta estradiol (E2) and progesterone (P4) concentrations during normal and abnormal cycles were also evaluated. **Methods:** Total of 447 MC from 93 Mfl were evaluated. Number of MC evaluated for each female ranged from 2 to 7 (mean = 4.8). Mean value of the first two cycles was treated as baseline cycle (BC). Following cycles (Study Cycle, SC) were compared with BC. Samples for E2 and P4 were collected every 2 to 7 days during MC, and were analyzed using ELISA method. Profiles of E2 and P4 during normal (22 to 38 days) and prolonged (50 to 92 days) MC were compared.

Results: MC was variable within and between females. Mean and median cycle lengths of all individual cycles was 30.8 and 30 days (range 17 to 63 days), and approximately 94% of the MC were between 22 to 38 days. Approximately 90% of SC was within ±20% of the BC. The peak E2 and P4 concentrations were observed on menstrual days (MD) 13 and 23, respectively, in normal MC. These peaks delayed in prolonged MC (MD34 and MD59, respectively). Interval between E2 and P4 peaks, and interval between E2 peak and the next menses were extended in prolonged cycles while change in interval between P4 peak and next menses was minimal. **Conclusion:** Normal range of menstrual cycle in cynomolgus monkey was from 22 to 38 days. Over time, the normal menstrual cycle can fluctuate up to 20% from the baseline cycle in individual animals. In prolonged cycles, occurrences of peak E2 and P4 concentration also delayed but the interval between peak P4 and onset of the next menses was not affected.

PS 1062 ESTROGENIC IMPACTS OF A STANDARD RODENT CHOW DIET ON RAT REPRODUCTION AND DEVELOPMENT.

H. Aoyama[1], K. L. Takahashi[1], N. Endo[1], A. Sato[1], M. Araki[1], M. Harigae[1], M. Mitsutani[1], C. Urakawa[1], T. Nakamura[1], N. Sakai[1], K. Sakasai[1], S. Teramoto[1], F. Horio[2], A. Murai[2], M. Kobayashi[2] and H. Hojo[1]. [1]*Toxicology Division, Institute of Environmental Toxicology, Joso, Japan and* [2]*Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan.* Sponsor: M. Kuwagata.

Standard rodent chow diets usually contain a considerable amount of phytoestrogens and are suspected of having unexpected impacts on reproduction and development of laboratory rats and mice. The present study aimed at examining possible estrogenic properties of standard chow diets on reproductive and developmental phenotypes of intact experimental animals, in which inbred Wistar Kyoto (WKY/NCrlCrlj) rats, 24/sex/group, were fed a purified diet (AIN-93G) containing no detectable levels of phytoestrogens or a phytoestrogen-rich standard rodent chow diet (MF) for two successive generations. Parental rats exhibited no significant differences between the two groups in such parameters as mating, fertility and delivery indices. However, the numbers of implants and/or newborns per females, as well as the weights of seminal vesicles and prostates of males in the MF group were significantly lower than those in the AIN-93G group in both generations. Significant differences were also found in developmental parameters of F1 and/or F2 offspring between the two groups: female weanlings in the MF group had significantly heavier uterus than those in the AIN-93G group, vaginal opening of females was slightly accelerated in the MF group, and preputial separation of males was clearly delayed in the MF group. Most alterations in the MF group were similar to those observed in the animals treated with estrogenic compounds such as 17-β estradiol, ethinyl estradiol and methoxychor. As the total concentration of phytoestrogens was more than 400 ppm in the MF diet while less than the detectable limit in the AIN-93G diet in the present study, the differences between the two groups are suggested to be due, at least in part, to estrogenic effects of phytoestrogens in the standard rodent chow diet.
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PS 1063 EFFECTS OF NEONATAL BISPHENOL-A EXPOSURE ON PERIPUBERTAL DEVELOPMENT OF FEMALE RAT HYPOTHALAMUS.

S. M. Losa, K. L. Todd, K. McCaffrey, M. Radford and H. B. Patisaul. *Biology, North Carolina State University, Raleigh, NC.*

We and others have previously found that Bisphenol A (BPA) exposure during the neonatal period, a critical window of sexually dimorphic hypothalamic differentiation in the rat, can alter the timing of female pubertal onset, induce irregular or premature cessation of the estrous cycle, and ovarian malformations in adulthood. It is now well recognized that hypothalamic neurons which produce the kisspeptin family of peptide hormones (KISS neurons) are critical for initiating puberty and maintaining estrous cyclicity. Here we explored the hypothesis that neonatal BPA exposure disrupts the sex specific density of KISS neuronal fibers in the antereoventral periventricular nucleus (AVPV) and arcuate nucleus (ARC), and the ontogeny of KISS inputs on gonadotropin releasing hormone (GnRH) neurons. To test this we used a transgenic strain of Wistar rats where their GnRH neurons produce green fluorescent protein (GFP). Pups were exposed via subcutaneous injection to vehicle, 17β estradiol (E2), 50μg/kg BPA (the current reference dose) or 50mg/kg BPA (the current LOAEL) daily from the day of birth (post natal day (PND) 0) through PND 3 and then cohorts were sacrificed on PNDs 17, 21, 24, 28, and 33 (5-8 animals per age per exposure; males collected on PNDs 21 and 33) via pericardial perfusion. Vaginal opening, body weight, serum luteinizing hormone levels and ovarian histology were also assessed in the females. KISS fiber density and contacts on GnRH neurons were quantified using immunohistochemistry and confocal microscopy. Vaginal opening was slightly advanced by E2 and the low dose of BPA. By PND 33, E2 and 50mg/kg BPA exposed females had AVPV KISS fiber density more typical of males and decreased numbers of KISS contacts on GnRH neurons. E2 additionally resulted in decreased ARC KISS fiber density at all time points. These findings suggest that neonatal BPA exposure can defeminize the female hypothalamus in the peripubertal window.

PS 1064 IMPACT OF NEONATAL BPA EXPOSURE ON SEXUALLY DIMORPHIC GENE EXPRESSION IN THE POSTNATAL RAT HYPOTHALAMUS.

J. Cao, J. A. Mickens, K. McCaffrey, K. L. Todd and H. Patisaul. *Biology, North Carolina State University, Raleigh, NC.* Sponsor: G. LeBlanc.

In rats, the first few days of neonatal life are a critical time of sex specific hypothalamic differentiation. It has been hypothesized that exposure to Bisphenol A (BPA) during critical windows of development, interferes with sexual differentiation of the rodent hypothalamus. To test this, we first mapped the temporal and sexually dimorphic neonatal expression profiles of the two major estrogen receptor isoforms (ERα and ERβ) in the anterioventral periventricular nucleus (AVPV), medial preoptic area (MPOA), ventromedial nucleus (VMN) and arcuate nucleus (ARC), using in situ hybridization. ERα expression was generally higher in females (Long Evans rat) through postnatal day (PND) 19 except in the ARC. AVPV ERβ expression was higher in males at birth, then declined and was ultimately higher in females on PND 19. VMN ERβ levels were higher in females until PND 19. Thus, we determined that hypothalamic sex differences can be pronounced at birth but transient, and region specific. We next tested the hypothesis that exposure to BPA during this period would alter this sexually dimorphic gene expression profile. Long Evans neonates were exposed to vehicle, 10 μg estradiol benzoate (EB), 50 mg/kg BPA or 50 μg/kg BPA by subcutaneous injection on the first three days of life (PNDs 0, 1 and 2). Pups were sacrificed and the brains collected on PNDs 4 and 10 (6-8 per age per sex per exposure group). EB exposure significantly decreased ERα expression levels in the female AVPV and the MPOA of both sexes. In contrast, BPA exposure produced the opposite effect in the AVPV and enhanced ERα expression in both sexes, augmenting the sex difference on PND 4. No effect of BPA was observed on MPOA ERα expression in either sex. Our results indicate that BPA may interfere with sexually dimorphic ER expression during the neonatal critical period. Moreover, the opposite effect of EB and BPA on ERα expression indicates that BPA may disrupt neuroendocrine differentiation by a mechanism other than simply binding to ERs and acting as an estrogen mimic.

PS 1065 BENZO(A)PYRENE-INDUCED OVARIAN DYSFUNCTION RESULTS FROM SUPPRESSED E_2 SYNTHESIS.

A. E. Archibong[1], A. Ramesh[2], M. S. Niaz[2] and D. J. Alcendor[3]. [1]*Physiology, Meharry Medical College, Nashville, TN,* [2]*Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN and* [3]*Microbiology and Center for Health Disparities Research in HIV, Meharry Medical College, Nashville, TN.*

Epidemiological data suggest that smokers have delayed conception and premature ovarian failure leading to early menopause. The toxic effects of cigarette smoke on ovarian function are difficult to assess in as much as cigarette smoke contains more than 4,000 chemicals. Benzo(a)pyrene (BaP), an endocrine disruptor that perturbs ovarian function, is one of the major components of cigarette smoke. The objective of this study was to study the mechanism by which BaP perturbs normal functioning of the ovary. Female F-344 rats with regular four-day estrous cycles were used in this study. Rats were administered BaP (5mg BaP/kg) or vehicle (tricaprylin; control) by oral gavage for 30 days. A week after the onset of gavaging, daily vaginal smears were conducted to determine the ability of BaP-exposed rat to cycle continuously. At the end of the treatment regimen, blood samples were collected from rats at proestrus for the determination of serum estradiol-17β (E_2), LH and FSH concentrations. Subsequently, ovaries were harvested post CO_2 asphyxiation for determining the expression of pro-inflammatory biomarkers. BaP significantly increased estrous cycle length by approximately 3 days. Serum concentrations of E_2 and LH were decreased ($P < 0.05$) while those of FSH were increased ($P < 0.05$) at proestrus compared with those of their control counterparts. Furthermore, some pro-inflammatory biomarkers were significantly elevated in BaP-exposed versus control rats. We conclude that the reduced response of the ovaries to pituitary FSH stimulation, based on reduced E_2 secretion at proestrus, is influenced by pro-inflammatory cytokines. As a consequence, increases in E_2-stimulated LH secretion and normal E_2-regulated estrous cycle length could not be realized. This study was supported by the USPHS grants, G12RR03032, U50ATU3989-48-06, 1U54HD0431501-09, RO1 HD020419-19S1, 1S11ES014156-05, 1RO1CA142845-01A1, and 1RO3CA130112-01.

PS 1066 *IN UTERO* EXPOSURE TO DIBUTYL PHTHALATE ALTERS GENE EXPRESSION LEVELS IN THE FETAL RAT FORESKIN.

J. Pike, S. McCahan and K. Johnson. *Nemours Biomedical Research, Alfred I. duPont Hospital for Children, Newark, DE.*

Fetal phthalate exposure during a critical window of rat development has been shown to result in improper formation of the male reproductive system, including the phallus, through disruption of testosterone production. Gene expression analysis of the foreskin could provide a translational biomarker correlating with fetal phthalate exposure and subsequent adverse effects. Fetal rats exposed daily to 100 or 500mg/kg of the endocrine disruptor dibutyl phthalate (DBP) from gestational day (GD) 16-20 were sacrificed on GD20. Significant decreases in fetal anogenital distance (AGD) and testis testosterone levels confirmed endocrine disruption at the 500mg/kg DBP exposure level, but not the 100mg/kg level. A significant increase in the presence of multinucleated germ cells (MNGs) was observed at both exposure levels. Global gene expression was assessed in male foreskin total RNA with Illumina RatRef-12 Expression BeadChips. Differentially expressed genes were identified using LIMMA statistical analysis with a false discovery rate (FDR) adjustment ($p<0.05$). While analysis did not identify differentially expressed genes at the 100mg/kg DBP exposure level, there were 216 probes that differed between the 500mg/kg DBP exposure and the control group. Gene expression changes for the cholesterol biosynthesis regulator *Insig1* were confirmed by real-time RT-PCR analysis. These data are indicative that in utero phthalate exposure affects gene expression levels in male fetal rat foreskin.

PS 1067 INHALED CADMIUM OXIDE (CDO) NANOPARTICLES (NP) REACHES THE PLACENTA AND DECREASES PREGNANCY INCIDENCE AND GROWTH RATES IN NEONATAL OFFSPRING IN FEMALE MICE.

L. K. Rosenblum, J. L. Blum, J. Q. Xiong and J. T. Zelikoff. *College of Medicine, New York University, Tuxedo, NY.*

Advances in synthetic chemistry have led to the development of NP that are being used everywhere from computers to medicine. As an industry, nanotechnology is expected to exceed $1 trillion by 2015. One NP being used industrially is CdO. Along with its use in the manufacture of quantum dots, industries using CdO NP also include plastics, fiber and textiles, and research is being pursued to determine other uses in biosciences. To date, no studies have examined the potential toxicity of CdO NP to female reproductive health. Therefore, a study was carried out using timed-pregnant CD-1 mice to determine if inhaled CdO NP could reach the uterus or placenta and cause deleterious effects. Two separate experiments were performed. Pregnant mice were exposed either every other day to 100 μg CdO/m^3 (2.5 hours/day) or every day to 220 μg CdO/m^3 for 2.5 hours. The exposures started at 4.5 days post coitus (dpc) and ended at 16.5 dpc; at 17.5 dpc, the fetuses and placentas were collected, measured, and weighed. Some mice from the higher concentration study were also allowed to give birth and neonates were weighed daily through weaning. Inhaled CdO translocated to the uterus and placenta, but did not reach the fetus. At 220 μg CdO/m^3, a 20-29% decrease in pregnancy rate was observed which may be correlated with a delay in maternal weight gain seen just following implantation. There was also a significant increase in placental weight and decrease in length of fetuses from dams exposed to CdO while no change in fetal weight was seen. Newborns of CdO-exposed dams had decreased growth rates, with growth milestones being delayed by up to three days, due possibly to deficiencies in milk quality or quantity prior to five days-of-age, or Cd being present in the milk. Taken together, exposure of dams to inhaled CdO NP seems to not only have an effect on conception, but also on the growth of their unborn and neonatal offspring.

PS 1068 A MECHANISTIC APPROACH TO THE ROLE OF TRIBUTYLTIN IN THE DEVELOPMENT OF MALE-TYPE GENITAL ORGANS IN FEMALE QUEEN CONCH (*STROMBUS GIGAS*).

O. S. Phillip[2, 1], K. Thomas[3], C. O'Neal[1] and W. Gray[2]. [1]*Marine Biology, H. Lavity Stoutt Community College, Road Town, Tortola, BVI, United Kingdom,* [2]*Chemistry, Southern University and A&M College, Baton Rouge, LA and* [3]*Center for Environment, Fisheries and Aquaculture Science, Burnham on Crouch, Essex, United Kingdom.*

The use of organotin compounds in the mass production of antifouling biocides, fungicides, pesticides and wood preservatives have resulted in the release of large amounts of organotins into the environment. Studies show that the extensive use of these lipophilic compounds have proven to be highly toxic to marine organisms. Tributyltin (TBT), a prototypical organotin compound, is known to induce the superimposition of male-type genital organs (penis and vas deferens) on female gastropods (termed imposex), even at very low concentrations. However, the molecular mechanism by which TBT exposure results in the development of imposex in female gastropods is not clearly defined. The present study utilizes a highly commercialized gastropod model to test the hypothesis that exposure to TBT results in the masculinization of the female queen conch, *Strombus gigas*, in the coastal marine environment of the British Virgin Islands. To mechanistically establish the basis of TBT-induced imposex water and conch samples were obtained from three sites around the island of Tortola, BVI. Conch samples were histopathologically analyzed to confirm the existence of male-type genital organs on female conchs. This was followed by gas chromatography with flame photometric detection of both the sea water and conch samples. Additionally, western blot analysis of the Sp1/3 transcription factors was done to investigate the effect of TBT on genital organ development. The results of this study show: Queen Conch samples obtained from boating areas had a high incidence of imposex; chromatography analysis showed the accumulation of TBT in various tissues; western blot analysis exhibit a downregulation of Sp3 protein in the TBT-exposed conch samples. These results strongly suggest that TBT induce the development of imposex in female queen conch in the coastal waters of the British Virgin Islands.

PS 1069 TOXICOGENOMIC RESOLUTION OF EARLY BIOMARKERS FOR LATER LIFE DISEASE ONSET RELATED TO CANCER, PRO-INFLAMMATORY DISORDERS, AND OBESITY IN MICE EXPOSED *IN UTERO* TO MAINSTREAM CIGARETTE SMOKE.

J. L. Blum[1], K. Sexton[2], C. Hoffman[1], K. BéruBé[2] and J. T. Zelikoff[1]. [1]*Environmental Medicine, New York University School of Medicine, Tuxedo, NY and* [2]*School of Biosciences, Cardiff University, Wales, United Kingdom.*

A toxicogenomics study was undertaken to evaluate lung gene changes in juvenile mouse offspring exposed prenatally to mainstream cigarette smoke (MCS; versus clean air). Such early fetal insults bring about certain cellular/molecular changes in offspring that can serve as early powerful predictors/biomarkers of chronic illnesses later in life. Timed-pregnant mice were exposed daily by inhalation to unfractionated MSC (4 hr/d, 5 d/wk) from gestation day 1 until parturition at a concentration equivalent to smoking < 1 pack/d (total suspended particulate matter, carbon monoxide and carboxyhemoglobin = 15 mg/m3, <25 ppm, and <5%, respectively). Lungs from pregnant dams exposed to MCS or filtered air and 5-wk-old female offspring were perfused and preserved by storing in RNALater. RNA was isolated and accessed by Bio-analyzer, Cy3/Cy5 labeled and hybridized to the Agilent mouse genome chip (41,000+ genes/transcripts). Common genes were extracted and subjected to pathway analysis to identify underlying disease processes. Only 38 genes were significantly altered (fold-change >2, Mann-Whitney uncorrected $p<0.05$) in MCS exposed dams (21 up-regulated and 17 down-regulated) versus 1934 altered genes in female offspring (451 up-regulated and 1483 down-regulated). Communal biological pathways were shown to be related to cancer, inflammation and obesity, which were significantly higher in female offspring (9166) versus dams (587), signifying a potential sexual dimorphism for these specific disease phenotypes. Historically, adverse health effects linked to CS have been based on causal associations determined by epidemiology. Today, toxicogenomics provides robust empirical biomarker predictors of disease, enabling therapeutic and clinical interventions. Supported by Institute for Science and Health.

PS 1070 FOXO1 ASSOCIATED P27KIP1 DECREASE IS ESSENTIAL FOR FENVALERATE-INDUCED CELL GROWTH IN HUMAN UTERINE LEIOMYOMA (UTLM) AND SMOOTH MUSCLE CELLS (UTSMCS).

X. Gao, L. Yu, L. Castro, A. B. Moore, M. I. Sifre, C. D. Bortner and D. Dixon. *NIEHS, Research Triangle Park, NC.*

Our previous data showed that Fenvalerate (Fen) promotes cell proliferation in human UtLM cells and UtSMCs by shortening the G0-G1 phase and enhancing G1 to S phase progression through a non-estrogen receptor (α/β) mechanism. The purpose of the current study was to explore other possible molecular mechanisms associated with this effect. Real-time RT-PCR, western blot (WB) analysis and/or confocal immunofluorescence microscopy were used to detect the expression of the cyclin-dependent kinase inhibitor, p27kip1 and its association with FoxO1. In UtLM cells and UtSMCs, FoxO1 and p27kip1 mRNA levels were significantly diminished by 10 μM Fen from 10 min to 24 h compared to controls. A reduction in intensity of p27kip1 and FoxO1 positive staining was found in both cell types treated with Fen at 24 h compared to vehicle controls. This trend was further confirmed by WB analysis for both p27kip1 and FoxO1 in Fen-treated UtLM cells and

UtSMCs. Phosphorylation of FoxO1 was largely abolished by 24 h following Fen administration, and Fen induced increased association of FoxO1 and p27kip1 as evidenced by immunoprecipitation in both cell lines. Overexpression of doxycycline-regulated adenovirus p27kip1 (Ad.p27kip1) resulted in inhibition of proliferation and markedly reduced mRNA and protein levels of CDK2 in both cell lines. Fen was found to restore these effects, including cell growth with significantly increased percentages of cells in S phase, but decreased percentages in G0-G1 phase. Consequently, our results indicate that down-regulation of p27kip1 by Fen in UtLM cells and UtSMCs is associated with decreases in FoxO1. This suggests that FoxO1/p27kip1 could be a potential target pathway for Fen-induced cell proliferation through the regulation of CDK2 and cell cycle progression, and may possibly play a role in the pathogenesis of fibroids following environmental exposures.

PS 1071 INVESTIGATING THE IMPACT OF CHRONIC HERBICIDE EXPOSURE ON SEXUAL DEVELOPMENT IN ZEBRAFISH.

M. M. Corvi[1], K. A. Stanley[1], J. K. LaDu[1], T. S. Peterson[3], S. W. Feist[2], M. L. Kent[2, 4] and R. L. Tanguay[1]. [1]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR,* [2]*Weymouth Laboratory, Centre for Environment, Fisheries & Aquaculture Science, Weymouth, United Kingdom,* [3]*Microbiology, Oregon State University, Corvallis, OR and* [4]*Environmental Health Sciences Center, Oregon State University, Corvallis, OR.*

The potential effects of the herbicide atrazine (ATZ) on aquatic species are highly controversial; some studies have reported that exposure to low ATZ concentrations adversely impacts gonadal development and/or sexual differentiation in amphibians and fish while others do not. A chronic study was performed investigating the potential effects of ATZ on zebrafish (Danio rerio). Larval fish were exposed to nominal concentrations of 0, 0.1, 1, 10 μM ATZ, or 1 nM 17beta-estradiol (E2) from 17-130 days post-fertilization (dpf). Treatment groups consisted of eight replicate tanks with a total of 10 fish per tank. At study termination, body measurements were taken; then fish were histologically processed. Slide sections for each fish were examined to assign sex and evaluate potential impacts of E2 or ATZ on gonadal development. Exposure to E2 resulted in a significantly higher proportion of females to normal, mature fish, a greater percent of abnormally developed male fish, and fish lacking differentiated gonadal tissue. In contrast, ATZ exposure did not influence the percentages of female or male fish when compared to control. Estradiol tanks had significantly larger male fish while 10 μM ATZ exposures resulted in smaller zebrafish than their control counterparts. The study indicates that exposure to 0.1, 1. 10 μM ATZ does not significantly impact zebrafish gonadal development or sexual differentiation.

PS 1072 CIGARETTE SMOKE CONDENSATE INHIBITS FOLLICULAR DEVELOPMENT, OOCYTE MATURATION, AND DYSREGULATES STEROIDS SYNTHESIS *IN VITRO*: IMPLICATIONS FOR HUMAN FECUNDITY.

J. Sadeu and W. G. Foster. *Department of Obstetrics and Gynecology, McMaster University, Hamilton, ON, Canada.*

Decreased fertility and poorer response to ovulation induction have been reported in women who smoke compared to non-smokers. The cytotoxic effects and underlying mechanisms of action of cigarette smoke on ovarian function are poorly defined. We therefore hypothesize that cigarette smoke condensate (CSC) will affect follicular development, steroidogenesis, cumulus cell differentiation and oocyte maturity. Isolated mouse follicles (100-130 μm) were cultured for 13 days (D13) in the absence (controls) or presence of increasing CSC concentrations (30-130 μg/ml). Follicular development and viability were recorded prior to refreshment on days 4 (D4), 8 (D8) and 12 (D12) of culture. On D12, follicle survival was determined and preovulatory follicles stimulated with hCG and EGF. Following 18h (D13) after stimulation, cumulus cell-oocyte complexes, oocyte diameter and nuclear maturation were determined. Steroid output in spent media was measured (ELISA) on D8 and D12 (E2), and D12 and D13 (P4). Follicular development significantly ($p<0.05$) decreased at the preantral (D4; 130 μg/ml) and antral (D8 and D12) stages in CSC-treated groups. In addition, follicle survival decreased in a dose dependent manner and significant in the highest treatment (90 and 130 μg/ml) groups. Oocyte diameter was similar between controls and CSC-exposed follicles, while oocyte maturation was significantly decreased in CSC-treated groups. E2 output decreased significantly at the preantral (D4; 90 and 130 μg/ml) stage, whereas P4 increased significantly prior to ovulation (D12), and decreased significantly on D13 in all treatment groups. We conclude that exposure to CSC disrupts follicular development, decreases follicle survival, disregulates steroidogenesis (E2 and P4), alters cumulus cell differentiation, and delays or blocks oocyte meiotic maturation. Thus, we suggest that cigarette smoking impairs human fecundity through direct adverse effects on folliculogenesis and oocyte maturation. Funding for this project was provided by CIHR.

PS 1073 TREATMENT OF *CAENORHABDITIS ELEGANS* WITH GLYPHOSATE SUGGESTS DEVELOPMENTAL AND REPRODUCTIVE TOXICITY.

M. B. Johnson and V. A. Fitsanakis. *Biology, King College, Bristol, TN.*

Herbicides are widely used in both agricultural and residential areas; therefore, pesticide users and their family members may be routinely exposed to these potentially harmful chemicals. Epidemiological studies suggest that pesticide exposure may increase a woman's risk of spontaneous abortions. A significant gap in the literature exists as to whether these observations may be due to exposure to glyphosate-containing herbicides. To further examine this question, we used the model organism *Caenorhabditis elegans* to test our hypothesis that exposure to the glyphosate-containing herbicide TouchDown (TD) results in decreased number of offspring in wild-type (N2) worms. Based on concentrations (LC_{50} or LC_{75}) from previous data, worms were chronically (24 hours) exposed to TD. In the first study, eggs, isolated from gravid worms with a hypochlorite/hydroxide solution, were exposed to TD. Subsequently, the number of successfully hatched worms was counted to determine whether TD adversely affected embryo viability. One-way ANOVA indicated a statistically significant decrease ($p < 0.001$) in the number of hatched eggs in treated compared to control worms. In our second study, we initially treated gravid hermaphrodites (F_0) with TD, and then transferred randomly selected offspring (F_1) from control or treated populations, respectively, to new nematode growth plates. The F_1 generation was allowed to reproduce in the absence of further treatment. F_2 generation worms were then counted to determine whether the reproductive capacity of the F_1 generation had been diminished. A statistically significant decrease ($p < 0.05$) was observed in the number of F_2 worms only from F_0 worms treated with the LC_{75} concentration (9.8% glyphosate). These data suggest that developmental (decreased hatching) and reproductive (decreased number of viable offspring) toxicity may result from exposure of *C. elegans* to TD. These results indicate the importance of future studies in higher organisms to provide a better understanding of the developmental and/or reproductive toxicity of glyphosate-containing pesticides.

PS 1074 MITOGENIC AND APOPTOTIC INTERACTIONS OF BENZO[A]PYRENE IN JEG3 HUMAN TROPHOBLAST CELLS: IMPLICATIONS FOR INTRAUTERINE GROWTH RESTRICTION.

I. N. Olomu and B. V. Madhukar. *Pediatrics and Human Development, Michigan State University, East Lansing, MI.*

Maternal cigarette smoking is associated with adverse birth and reproductive outcomes including preterm delivery, intrauterine growth restriction and placental abnormalities. The precise mechanisms of these outcomes remain unclear. In the present study we investigated the cellular interactions of benzo[a]pyrene (B[a]P) a major component of cigarette smoke in JEG3, a human trophoblast cell line. Exposure of JEG3 cells to 100.0 nM B[a]P caused a modest but significant increase in cell proliferation. To gain insight into the cellular pathways modulated at this concentration of B[a]P, we examined the activation of mitogen activated protein kinases (ERK-MAPKs) and epidermal growth factor receptor (EGFR) as well as changes in cell cycle regulating proteins. Western immunoblot analysis of total cellular proteins from JEG3 cells treated with 100.0 nM B[a]P showed an increase in the, 1. Activation state of ERK-MAPKs and EGFR, and 2. Level of cyclin D1. In contrast to the increase in the proliferation of JEG3 cells at the low concentration, B[a]P inhibited cell proliferation at high concentrations (10.0 or 40.0 μM). Such an inhibition appeared to be due to an increase in apoptosis of the cells as judged by increased (20-25%) number of acridine orange stained cells and was further confirmed by TUNEL staining. cDNA microarray analysis of JEG3 cells treated with 100.0 nM or 40.0 μM B[a]P showed an increase in a number of cell growth associated genes at 100.0 nM B[a]P. At 40.0 μM B[a]P increased the expression of apoptotic genes such as caspases and decreased the levels of growth associated genes. Taken together these results suggest that exposure of human trophoblast cells to B[a]P at concentrations that may be present in the placenta is associated with proliferation. Enhanced trophoblast proliferation may in part explain the trophoblast hyperplasia often observed in the placenta of mothers that smoke cigarettes during pregnancy.

PS 1075 CARBON MONOXIDE TOXICITY AND THE MATERNAL-FETAL ENVIRONMENT.

T. Dodd-Butera[1] and M. Broderick[2]. [1]*Nursing, California State University San Bernardino, College of Natural Sciences, San Bernardino, CA and* [2]*California Poison Control System, San Diego, CA.*

Carbon monoxide (CO) exposure to the maternal-fetal environment has been linked to preterm delivery, malformation, and fetal demise. National Poison Data System (NPDS) Information reports approximately 35,000 pregancy-related cases in the United States, with California cases accounting for approximately 10% during years 2004-2007. This was an IRB-approved retrospective review of 3805 pregnancy-related cases, with 77 cases of CO exposure; recorded from the California Poison Control System (CPCS), years 2004-2007. In cases of exposure where maternal age was known, all but 3 cases were 20 years and older. Carboxyhemoglobin (COHb) levels were done on 22 of the CO-exposed cases, with oxygen therapy administered in 13 cases, and Hyperbaric oxygen (HbO) performed in 3 cases. COHb levels in two of the three cases administered HbO were recorded at 21% and 25% and both were in the 2nd trimester. The third case was in the first trimester, with a COHb level of 13%. Initial short-term follow-up indicated maternal and fetal well-being, but follow-up to birth was not noted in any of the cases. Previous toxicology studies in animal models have indicated adverse cardiac and neurologic potential post-exposure to CO during gestation. A recommendation for a revised follow-up clinical toxicology protocol unique to pregnancy could be considered by poison centers, in order to improve outcome measurements and add to the body of scientific knowledge regarding in utero exposures in humans; particulary those substances with teratogenic potential. This would improve the quality of toxicology data for human exposures to CO during pregnancy.

PS 1076 CHARACTERIZATION OF A NOVEL SERIES OF ENDOTHELIN-A RECEPTOR ANTAGONISTS IN THE SETTING OF INFECTION ASSOCIATED PRETERM BIRTH.

N. S. Olgun, H. J. Patel, R. A. Stephani and S. E. Reznik. *Pharmaceutical Science, St. John's University, Jamaica, NY.*

Clinically defined as any birth occurring prior to 37 weeks' of gestation, preterm birth (PTB) accounts for approximately 13% of all live births in the United States each year and is a leading cause of infant mortality. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide which is upregulated by inflammatory cytokines and is capable of causing an increase in myometrial tone. In the setting of infection in pregnancy, ET-1 has been shown to be up-regulated, ultimately leading to PTB. In our previous work, we have shown that our novel series of 1-3-6-trisubstituted-2-carboxy-quinol-4-ones act as selective Endothelin-A receptor antagonists (ET A-RAs). In particular, HJP-272, the prototype compound, binds to the ET A receptor with an IC 50 value of 70.1nM in a non-competitive manner. We have also shown that in the animal model, HJP-286, the n-propyl analogue, and HJP-272 successfully shut down PTB. Concern for the clinical utility of these compounds as tocolytic agents is based on their potential teratogenic effects. Therefore, in the current work, we investigate whether or not there are any chronic histological changes taking place in the animal model following exposure to these agents. C57Bl/6 mice are injected with either phosphate buffered saline, vehicle(dH20 with KOH and K2CO3), or HJP-272, HJP-286 or HJP-278 (n-butyl analogue). Animals are observed for ten days and then sacrificed. All vital organs are harvested, processed, and observed using light microscopy to assess specific organ toxicity. Previous acute toxicity studies in which the animals were sacrificed after 24 hours showed no difference between control and treated animals. From these studies, we can conclude that not only do our compounds have a high affinity for the ET A receptor, but they also prevent PTB at doses in which we see no toxicity in the mother. It is our hope that these compounds may one day affect the way pregnant women presenting with PTB are treated, as there is currently no FDA approved therapy for this very important clinical disorder.

PS 1077 AFFECTS OF ALCOHOL CIRRHOSIS AND DIABETES ON DRUG TRANSPORTER EXPRESSION IN HUMAN LIVER.

V. R. More[1], Z. Lu[2], N. Cherrington[3] and A. Slitt[1]. [1]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI,* [2]*Statistical Consulting Laboratory, University of Arizona, Tucson, AZ and* [3]*Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Transporters are important for the uptake and excretion of many drugs and environmental chemicals in the liver. While alterations in the expression of these transporters would have profound effect on the fate of numerous compounds within the body, limited data exists regarding the effect of important types of liver injury on transporter expression in human liver. Diabetes and alcohol-induced liver cirrhosis are two major liver injuries that are present in the American population. Insulin levels are often elevated in alcoholism, with chronic alcoholism resulting in diabetes. Because there is a sizable population that presents with these diseases alone or in combination, it is important to determine the effect of diabetes, cirrhosis, or diabetes and cirrhosis on transporter expression in human liver. Therefore, drug transporter and nuclear hormone receptor mRNA expression in normal, diabetic, cirrhotic and diabetic-cirrhotic human livers was quantified. Target gene fluorescence intensity was normalized to hypoxanthine phosphoribosyl transferase-1 fluorescence intensity, and data was log transformed followed by MANCOVA. Cirrhosis significantly increased ABCC4, 5, ABCG2 and SLCO2B1 mRNA expression, and decreased SLCO1B3 mRNA expression in liver. Interestingly, in the livers of subjects with both diabetes and cirrhosis, transporter expression did not differ from controls, except for ABCC4. Cirrhosis and diabetes-cirrhosis increased NRF2 mRNA expression, and decreased PXR and FXR mRNA expression in liver compared to livers of normal subjects. Hierarchical cluster analysis indicated that expressions of ABCC2, 3 and 6; SLCO1B1 and 1B3; and ABCC4 and 5 are more closely related. Overall, significant cirrhosis, and cirrhosis-diabetes effects on transporter mRNA expression in human liver were revealed (The Rhode Island foundation, NIH 5K22ES013782).

PS 1078 PERINATAL BISPHENOL A (BPA) EXPOSURE ALTERS LIVER ATP-BINDING CASSETTE (ABC) TRANSPORTER EXPRESSION IN MICE.

A. C. Donepudi[1], M. Meloon[1], P. T. Sieli[3], C. S. Rosenfeld[2] and A. L. Slitt[1]. [1]*Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI,* [2]*Department of Biomedical Sciences, University of Missouri, Columbia, MO and* [3]*Bond Life Sciences Center, University of Missouri, Columbia, MO.*

BPA is a chemical produced in voluminous amounts in manufacturing of plastic and paper products. BPA exposure is pervasive. Perinatal BPA exposure alters gene expression in liver, but it is not clear whether these alterations are epigenetic or estrogenic in origin. ATP-binding cassette (Abc) transporters are efflux transporters, which efflux endogenous chemicals (e.g. conjugated hormones, leukotrienes) and xenobiotics. The purpose of this study was to investigate whether in utero (prenatal) BPA exposure affects liver Abc transporter expression. Female a/a (C57BL/6) mice were exposed to BPA through the diet (50 μg/kg or 50 mg/kg) or AIN93 Control diet with 7% corn oil from 2 weeks pre-mating with male Avy/a mice. BPA exposure was maintained through the breeding and lactation period. Pups were weaned on postnatal day (PND) 21 and maintained on the AIN93 control diet. Livers were collected from offspring after PND 135, total RNA was isolated, and Abcc 1-6, Abcg2, and Abcb1a was analyzed by qPCR. BPA perinatal exposure decreased hepatic Abcc2, Abcg2, and Abcb1a mRNA transporter expression in both male and female offspring at both concentrations. In contrast, BPA exposure increased Abcc4 expression in daughters exposed in utero to the 50μg/kg maternal diet BPA concentration, whereas BPA decreased Abcc4 expression in livers of sons at both concentrations. BPA exposure decreased Abcc6 expression in livers of sons, but not daughters. BPA perinatal exposure altered Abc transporter mRNA expression in liver. These data suggest that in utero BPA exposure might exert detrimental effects on offspring health by altering the expression of genes that are known to mediate the transport conjugated hormones and inflammatory mediators. (5R01ES016042, 3R01ES016042-02S1)

PS 1079 OPPOSING EFFECTS OF DIABETES AND PREGNANCY ON HEPATOBILIARY EFFLUX TRANSPORTER EXPRESSION.

A. L. Slitt[1], J. Xu[1] and L. M. Aleksunes[2]. [1]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI and* [2]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

Efflux transporters are plasma membrane proteins responsible for excreting chemicals and toxicants from the cell. In the liver, efflux transporters including the multidrug resistance-associated proteins (Mrps) are markedly induced in mice with diabetes. In contrast, other conditions such as pregnancy have been shown to decrease hepatic Mrp levels. Altered expression of efflux transporters influences not only the hepatic clearance of chemicals, but also susceptibility for hepatotoxicity. The purpose of the present study was to quantify the mRNA expression of hepatobiliary efflux transporters in pregnant, diabetic mice. Female C57BL/6 mice were treated with vehicle or streptozotocin (STZ, 50mg/kg/d for 4 days). Once STZ-treated mice exhibited elevated glucose levels (>250 mg/dl) for 2 weeks, they were mated with normoglycemic male C57BL/6 mice until plugged (designated as gestation day 0). Total RNA was isolated from livers of nonpregnant and pregnant female mice (gestation day 14) with normal or elevated glucose levels. Messenger RNA expression was quantified by qPCR. Blood glucose levels were similarly elevated in

nonpregnant and pregnant diabetic mice. Livers from nonpregnant, diabetic mice exhibited up-regulation of Mrp1, 2, 4, 5 and breast cancer resistance protein (Bcrp) mRNA that was attenuated in pregnant, diabetic mice. Mrp3 mRNA was similarly induced in livers of nonpregnant and pregnant diabetic mice. Mrp6 mRNA was decreased by pregnancy, regardless of glucose level. Collectively, these data suggest that pregnancy represses expression of efflux transporters in livers of diabetic mice. Additional studies are necessary to elucidate the transcriptional mechanisms underlying the opposing regulation of hepatobiliary transporters in response to diabetes and pregnancy. Supported by NIH ES016042, ES005022, and DK080774.

PS 1080 EFFECT OF TROGLITAZONE (TRO) ON ENDOGENOUS BILE ACID (BA) DISPOSITION IN RAT AND HUMAN SANDWICH-CULTURED HEPATOCYTES (SCH).

T. Marion[1], C. Perry[2], R. L. St. Claire III[2] and K. Brouwer[1, 3]. [1]*Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, NC,* [2]*Qualyst, Inc., Research Triangle Park, NC and* [3]*Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Inhibition of hepatic transport may mediate drug-induced liver injury by increasing intracellular accumulation of potentially toxic compounds such as BAs. Drug-induced changes in BAs may be indicators of hepatotoxicity. In this study, the endogenous BA pool was profiled in fresh rat and human SCH on culture day 4 using B-CLEAR® technology to compare species differences, and to determine effects of transporter inhibition on cellular BA accumulation and biliary excretion. Taurocholate (TCA), glycocholate (GCA), taurochenodeoxycholate (TCDCA), and glycochenodeoxycholate (GCDCA) were measured on day 4 in cells + bile canaliculi, cells, and medium of SCH by LC-MS/MS 24 h after addition of vehicle or 10 μM TRO, an inhibitor of the bile salt export pump (BSEP/Bsep). Total BAs (pmol/mg protein) were ~12-fold greater in human than rat SCH. Consistent with reports in vivo, ~90% of BAs in rat SCH were taurine-conjugated, while in human SCH, 99% of BAs were glycine-conjugated. Cellular accumulation of BAs in human SCH tended to decrease after TRO treatment. In rat SCH, TRO treatment decreased the biliary excretion index (BEI; [bile]/[cells + bile] calculated for each BA) of TCA 3%, GCA 12%, TCDCA 15%, and GCDCA 27%; in human SCH, TRO had no effect on the BEI of GCA, but decreased the BEI of TCA 43%, TCDCA 67%, and GCDCA 48%, consistent with inhibition of BSEP/Bsep. Species differences in inhibition of BA BEI by TRO may be due to differences in intracellular concentrations of TRO and/or metabolites after 24 h in culture. TRO decreased total BAs (cells + bile + medium) in human SCH by 19% compared to control, suggesting that TRO may affect BA synthesis pathways in addition to inhibiting BSEP. Together, these data demonstrate that BA synthesis and secretion occur in SCH similar to in vivo, and that TRO differentially affects BA disposition in rat and human SCH. Supported by NIH GM41935

PS 1081 EFFECTS OF SEEDING DENSITY AND DAYS IN CULTURE ON BILE ACID TRANSPORT AND Mrp4 EXPRESSION IN SANDWICH-CULTURED MOUSE HEPATOCYTES ARE NOT A RESULT OF OXIDATIVE STRESS.

B. C. Ferslew[1], X. Gu[2], B. Swift[3], J. E. Manautou[2] and K. L. Brouwer[1]. [1]*Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC,* [2]*Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT and* [3]*PKPD and Drug Metabolism, Allergan, Inc., Irvine, CA.*

Studies were undertaken to determine whether oxidative stress was responsible for changes in bile acid transport (B-CLEAR®-MO) and Mrp4 expression in sandwich-cultured mouse hepatocytes as a function of seeding density and days in culture. Freshly isolated mouse hepatocytes were seeded at $1.0x10^6$ cells/well (low density, LoD) or $1.5x10^6$ cells/well (high density, HiD) in modified DMEM on 6-well BioCoat™ plates and overlaid with Matrigel™. Gene expression was measured for Mrp4, Nqo1, Hmox1 and Hes1 using RT-PCR. Mrp4 protein was determined by Western blot and quantified by densitometry. ^{3}H-Taurocholate (TC) was used to assess transport function. Intracellular TC accumulation was reduced on day 4 compared to day 3 for both LoD and HiD. At LoD, TC *in vitro* biliary clearance ($Cl_{biliary}$, mL/min/kg, mean±SEM) and biliary excretion index (BEI, %, mean±SEM) were 38±5 and 30±2 on day 3, and 14±6 and 24±5 on day 4, respectively. At HiD, $Cl_{biliary}$ and BEI were 48±11 and 29±3 on day 3, and 38±15 and 43±5 on day 4, respectively. Mrp4 mRNA and protein expression were inversely related to seeding density. The influence of seeding density on TC transport and Mrp4 protein are consistent with previous observations from our laboratory. mRNA levels for oxidative stress genes Nqo1 and Hmox1 were not significantly affected by the culture conditions tested. Interestingly, Hes-1, a transcription factor involved in regulation of cell differentiation and proliferation, increased as a function of days in culture, but was not influenced by seeding density. Sandwich-cultured mouse hepatocytes seeded at LoD and HiD do not exhibit increased oxidative stress under the conditions investigated. Therefore, changes in bile acid transport and increased Mrp4 expression at LoD compared to HiD do not appear to be a function of oxidative stress.
Supported by NIH GM41935; DK069557

PS 1082 THE CELL SURFACE MARKER CD90 IDENTIFIES A SUBSET OF HEPATOCYTES PRIMED FOR INCREASED MATRIX PRODUCTION: IMPLICATIONS FOR ETHANOL-INDUCED LIVER FIBROSIS.

B. A. Hocevar, Z. Wang and L. M. Kamendulis. *Department of Environmental Health, Indiana University, Bloomington, IN.*

Hepatic fibrosis, initiated by excessive alcohol consumption, is a predisposing factor in the development of cirrhosis. Excessive production of extracellular matrix (ECM) leads to cirrhosis, loss of liver function, and eventually death. Deposition of ECM is stimulated by TGFβ, which is produced and activated during the fibrotic process. We hypothesize that TGFβ leads to epithelial to mesenchymal transition (EMT) in hepatocytes, which contributes significantly to ECM deposition seen in alcohol-induced liver fibrosis. Mice treated with 5% ethanol (w/v) for 2 weeks displayed ~2-fold increase in collagen deposition in the liver providing validation for our experimental model. To characterize the transdifferentiation of hepatocytes, cell surface marker expression was profiled in control and ethanol treated livers. We found that ethanol produced a 2.1-fold increase (6.9% to 14.3%) in CD90 cell surface expression and a 1.3-fold increase in CD44 expression while expression of CD133, CD29, CD326, and CD49f did not change. Differential gene expression in $CD90^+$ versus $CD90^-$ hepatocytes was assessed by microarray analysis. Reporters were selected as differentially regulated in a non-statistical approach yielding 2248 upregulated and 1413 downregulated reporters. Initial examination of genes differentially expressed in $CD90^+$ hepatocytes identified increased expression of genes involved in inflammatory processes (IL1α, IL1β, IL10, TNFα), leukocyte recruitment (CCL2, CCL3, CCL19) and genes regulating ECM (COL1A1, COL1A2, COL4A1, TIMP2, TIMP3). In addition, the $CD90^+$ population expressed markers identifying hepatic progenitor cells (CD44, Procr) and cells that have undergone EMT (ZEB2, Vimentin, PDGFRβ, TGFβ1). These results show that ethanol treatment leads to the expansion of the $CD90^+$ population. Characterization of the $CD90^+$ hepatocytes indicates that this population may be responsible for the excess matrix production that occurs during ethanol-induced fibrosis.

PS 1083 INVESTIGATING ER STRESS PATHWAY ACTIVATION IN LIVER CELLS AND TISSUE AS A TOXICOLOGICAL TOOL.

M. Lafleur, R. Hernandez and J. W. Lawrence. *Investigative Toxicology, Amgen, Thousand Oaks, CA.* Sponsor: S. Sawant.

One of the many functions of the endoplasmic reticulum (ER) is the proper folding and assembly of newly synthesized secretory and membrane proteins. ER stress occurs when there is an accumulation of unfolded proteins in the ER and can be elicited by normal physiological processes such as the differentiation of B cells into plasma cells, by pathological conditions such as oxidative injury or viral infections, or induced by a number of drugs or compounds that interfere with the protein folding machinery. An evolutionary conserved adaptive or protective response known as the unfolded protein response (UPR) is activated in response to ER stress. The UPR consists of 3 principal pathways that collectively, help to ameliorate the accumulation of unfolded proteins in the ER by increasing the protein folding machinery or eliminating unfolded proteins. If the UPR is unable to dampen the unfolded protein load in the ER, a set of cellular responses lead to apoptosis. We have characterized ER stress responses to various chemicals. ER stress inducers tested included a number of marketed pharmaceutical compounds as well as agents that interfere with the cellular protein folding machinery such as Tunicamycin and Thapsigargin. ER stress was assessed in liver cell lines as well as primary liver cells and tissue. We observed that pharmaceutical agents and classical ER stress inducers trigger the UPR pathways in a general manner; however, Atazanavir and Nelfinavir pathway responses were not similar. These data suggest that agents associated with ER stress may not all behave the same in vitro.

PS 1084 PROFILING IMPAIRED HEPATIC ENDOPLASMIC RETICULUM GLYCOSYLATION AS A CONSEQUENCE OF ETHANOL INGESTION.

J. Galligan[1], K. S. Fritz[2], H. Tipney[1], R. L. Smathers[2], C. T. Shearn[2], L. J. Hunter[1] and D. R. Petersen[2]. [1]*Pharmacology, University of Colorado Denver, Aurora, CO and* [2]*Toxicology, University of Colorado Denver, Aurora, CO.*

Chronic ethanol consumption remains a predominant cause of liver injury in the United States. The precise mechanisms underlying the progression of alcoholic liver disease (ALD) are poorly understood; however, alterations in post-translational

modifications (PTMs) have been observed. Glycosylation is the most abundant and diverse cellular PTM, affecting protein folding and activity. Using a mouse model of ALD, 2-D gel electrophoresis and hydrazide chemistry were employed to assess the hepatic endoplasmic reticulum (ER) glycoproteome. Gradual decreases in glycosylation were observed during a time-course of ethanol ingestion, with marked alterations occurring at week 6. To identify proteins associated with the observed changes in glycosylation, LC-MS/MS was employed. This approach yielded 30 proteins, with 50.0% of those proteins identified being novel identifications for this PTM. Of the proteins identified, triacylglycerol hydrolase (TGH) consistently displayed decreased glycosylation. Impaired glycosylation of TGH has been shown to result in increased cellular storage of lipids, consistent with the pathologies associated with ALD. To further elucidate processes and mechanisms behind the progression of ALD, all 30 proteins were subject to bioinformatic analysis. Of interest, terms associated with ER protein folding, unfolded protein response (UPR) signaling and esterase activity were found significantly enriched in our samples. Impaired protein folding and UPR signaling has been implicated in rodent models of ALD, however, precise mechanisms behind its induction are currently unknown. Interestingly, impaired ER glycosylation is reported to induce potent UPR activation when chemically induced with tunicamycin (TM). Consistent with the actions of TM, our data suggest that dysregulation of hepatic protein glycosylation may be an important initiating factor for impaired ER protein folding and altered lipid homeostasis associated with ALD. (R37 NIH/AA009300 & F31 AA018606).

PS 1085 CO-INVOLVEMENT OF MITOCHONDRIA AND ENDOPLASMIC RETICULUM IN ACROLEIN-INDUCED HEPATOCYTE APOPTOSIS.

D. Avila[1], J. Zhang[1], C. McClain[1, 2], S. Barve[1] and S. Joshi-Barve[1]. *[1]Department of Medicine/GI, University of Louisville, Louisville, KY and [2]Robley Rex Veterans Administration Medical Center, Louisville, KY.*

Background: Acrolein is a common environmental food and water pollutant and a major component of cigarette smoke. It is produced endogenously by the metabolism of several drugs and amino acids, and has also been identified as both a product and initiator of lipid peroxidation. Acrolein is known to be cytotoxic to many cell types including hepatocytes; however the molecular mechanisms underlying acrolein-induced hepatocyte death are not fully understood. Methods: Human hepatoma cells were used to measure acrolein-induced hepatocyte death and to study death-signaling pathways, including disruption of mitochondria, activation of endoplasmic reticulum stress (ERS), and the induction of apoptosis. Results and Conclusions: Acrolein exposure caused a decrease in intracellular antioxidant glutathione and a loss of survival of hepatic cells in a dose-dependent manner. Additionally, mitochondrial function was impaired dose-dependently. Acrolein activated several proteins in the ERS pathway including eIF2α and ATF4, and activated the kinases JNK and p44 MAPK. We postulate that acrolein initiates cross-talk between the ERS and mitochondrial death pathway, and contributes to hepatotoxicity and liver damage. Our study defines basic mechanisms involved in liver injury and hepatotoxicity due to reactive aldehyde pollutants such as acrolein.

PS 1086 FRUCTOSE INDUCES URIC ACID GENERATION AND MITOCHONDRIAL DYSFUNCTION IN LIVER CELLS DURING THE DEVELOPMENT OF METABOLIC SYNDROME.

M. A. Lanaspa, L. G. Sanchez-Lozada, C. A. Roncal-Jimenez, N. Li, C. Cicerchi and R. J. Johnson. *Renal Medicine, University of Colorado AMC, Denver, CO.*

Excessive dietary fructose intake may have an important role in the epidemics of obesity and diabetes as its intake parallels the development of these syndromes and because the ingestion of fructose can induce features of metabolic syndrome including hypertriglyceridemia and fatty liver. During its metabolism, fructose induces ATP depletion and further generation of uric acid. Here, we show that uric acid acts as a prooxidant molecule in hepatic cells exposed to fructose and that this increase in oxidative stress mediates the generation and accumulation of triglycerides. The mechanism whereby uric acid is able to induce oxidative stress involves the translocation and activation of the NADPH oxidase subunit NOX4 to the mitochondria. This way, in normal hepatic cells but not in NOX4 silenced cells; uric acid induces the generation of superoxide radical as determined with the fluorescent marker mitosox (200-fold increase as compared to non-exposed cells, $p<0.001$). Generated oxidative stress leads to decreased mitochondrial aconitase-2 (Aco2) activity (52 ± 5 % decrease, $p<0.05$) in the krebs cycle with further accumulation of citrate (3.3-fold increase, $p<0.01$). Released citrate from the mitochondria to the cytosol activates ATP citrate lyase (ACL, by its phosphorilation in serine 455) and fatty acid synthase (FAS, 2.3-fold increase) for triglyceride synthesis and accumulation (1.2-fold increase, $p<0.01$). Inhibition of ACL activity with radicicol (10 μM) or FAS activity with C75 (10 μM) blocks triglyceride accumulation in liver cells in response to fructose but not the generation of oxidative stress. Also, we show that blockade of uric acid synthesis with allopurinol inhibits fructose mediated triglyceride accumulation in hepatic cells. Therefore, since subjects sensitive to fructose also have phenotypes associated with hyperuricemia, this study could explain the mechanism whereby some subjects are more sensitive to the effects of added sugar.

PS 1087 DIBENZOFURAN EXPOSURE AFFECTS MITOCHONDRIAL PERMEABILITY TRANSITION INDUCTION.

C. M. Palmeira, F. V. Duarte, A. P. Gomes, A. M. Simões, A. T. Varela, J. S. Teodoro and A. P. Rolo. *Center for Neurosciences and Cell Biology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal.*

Dibenzofuran is an aromatic ether with properties and chemical structure similar to dioxins, listed as a pollutant of concern due to its persistence in the environment, potential to bioaccumulation and toxicity to humans. Our results indicate that mitochondria are probably a target in dibenzofuran-induced toxicity. A decrease in the mitochondrial phosphorylative efficiency elicited by dibenzofuran seems to be a critical event in dibenzofuran-induced impairment of cell function. Although mitochondrial transmembrane potential developed after succinate energization is not affected, the lag phase that occurs in the phosphorylative cycle (after addition of ADP) and precedes repolarization, is increased in mitochondria incubated with dibenzofuran. To further explore and understand the cause for the increased lag phases induced by dibenzofuran, we evaluated mitochondrial ATPase activity. No significant differences were found between mitochondria exposed to dibenzofuran and control. Since the decreased performance of phosphorylation in exposed mitochondria, was not correlated with a less efficient ATPase, further studies are being conducted in order to evaluate the mechanisms by which dibenzofuran affects mitochondria phosphorylative system. A possible interaction of dibenzofuran with the adenine nucleotide translocator (ANT) could be the cause for the decreased efficiency of ATP production. This interaction was evaluated using co-immunoprecipitation and titrations using bongkrekic acid (BkA) in transmembrane potential experiments. Mitochondrial permeability transition (MPT) induction in mitochondria incubated with dibenzofuran was also evaluated. Results show that this pollutant seems to prevent MTP induction, when compared with control mitochondria loaded only with calcium. Thus, although the impairment of ATP synthesis induced in hepatic mitochondria, data suggest that dibenzofuran mimic cyclosporine A or BkA to inhibit induction of the MPT.

PS 1088 REGULATION OF LIVER FLAVIN-CONTAINING MONOOXYGENASE 3 (FMO3) GENE EXPRESSION UNDER OXIDATIVE STRESS CONDITIONS.

S. Rudraiah[1], X. Gu[1], L. M. Aleksunes[2], S. Campion[1], R. N. Hines[3] and J. E. Manautou[1]. *[1]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, [2]Department of Pharmacology & Toxicology, Rutgers University, Piscataway, NJ and [3]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI.*

Flavin-containing monooxygenase 3 (FMO3) catalyzes the NADPH-dependent oxygenation of nitrogen-, sulfur-, and phosphorous-containing xenobiotics in human liver. The hepatic expression of FMO3 gene is highly variable and until recently it was thought to be uninducible. In this study, human and mouse FMO3 gene expression was evaluated in various in vivo and in vitro models of hepatic oxidative stress. Fmo3 mRNA levels were quantified over a 48 hr time course in male mice treated with toxic doses of acetaminophen (APAP; 400mg/kg) or alpha-naphthyl isothiocyanate (ANIT; 50 mg/kg). APAP induced Fmo3 mRNA levels by 19- and 1000-fold at 24 and 48 hrs, respectively. Similarly, Fmo3 mRNA levels increased by 2-4.5 fold between 2 and 12 hrs after ANIT treatment and returned to baseline levels by 24 hrs. Treatment of mice with carbon tetrachloride (25 μL/kg) and allyl alcohol (30, 60 mg/kg) did not change liver Fmo3 mRNA expression. FMO3 mRNA levels from APAP overdosed patients were similar to normal human livers. Reporter gene assays in HepG2 cells exposed to the pro-oxidants tert-butyl hydroperoxide and tert-butyl hydroquinone showed significant suppression of FMO3 reporter gene expression. Furthermore, over-expression of the transcription factor NFE2L2 resulted in marginal induction of FMO3 gene expression while over-expression of KEAP1, an NFE2L2 cytoplasm localization retainer, slightly decreased reporter activity. Over-expression of E2F1 dramatically suppressed, while ZFP161 induced FMO3 reporter gene expression. Collectively, not all toxicants producing oxidative stress alter FMO3 gene expression. However, toxic APAP and ANIT treatment in mice markedly increases Fmo3 gene expression. Here we also report that E2F1 and ZFP161, transcription factors associated with cell cycle, regulate FMO3 gene expression. Supported by NIH DK069557 and ES005022.

PS 1089 PFOA-INDUCED LIVER EFFECTS IN CD-1 AND PPAR-ALPHA KNOCK-OUT C57BL/6 MICE.

A. J. Filgo[1, 2], M. J. Hoenerhoff[2], G. E. Kissling[2], S. S. White[2], E. P. Hines[3], J. P. Stanko[2] and S. E. Fenton[2]. *[1]Toxicology, University of North Carolina Chapel Hill, Chapel Hill, NC, [2]CMPB, NTP, NIEHS, Research Triangle Park, NC and [3]NCEA, ORD, U.S. EPA, Research Triangle Park, NC.*

Perfluorooctanoate acid(PFOA) is a stable, synthetic C8 perfluoroalkyl acid, commonly used as a surfactant in oil and water repellents for fabric coatings, food storage, lubricants and fire extinguishing foams. PFOA is persistent in the environment, ubiquitous in human serum, and is hepatotoxic and carcinogenic in laboratory animals. Liver effects are believed to be dependent on peroxisome proliferator-activated receptor-alpha(PPAR-α) agonism, but the relevance of this mechanism in terms of human disease is controversial. In this study, wildtype(WT) and PPAR-α knockout(KO) C57BL/6 mice were gavaged with 0.1, 0.3, 0.6, 1 and 3 mg PFOA/kg body weight(BW) during all of pregnancy to evaluate for any PPAR-α independent liver effects in their offspring. Also, intact and ovariectomized(ovx) CD-1 mice were gavaged with 0.01, 0.1, 0.3, 1 and 5 mg PFOA/kg BW to evaluate for liver effects dependent on endocrine disruption. Offspring necropsy was conducted at 18 months; serum PFOA at that time was at background levels. Liver sections were examined by a NIEHS pathology review team. A significant dose-dependent increase in bile duct hyperplasia and hematopoietic cell proliferation was observed in the PPAR-α KO, and not WT mice treated with PFOA. Centrilobular hepatocyte hypertrophy was significantly increased in incidence, over their respective controls, in PFOA-exposed CD-1 and C57BL/6 mice, including the PPAR-α KO mice. Additionally, one or two hepatocellular adenomas were observed in all dosed groups of PPAR-α KO mice, but none were observed in WT mice. In the CD-1 (intact or ovx), a few adenomas were present in most groups, and the incidence in intact mice exposed to 0.3 mg PFOA/kg BW was significantly higher than in controls. Hepatocellular carcinomas were observed in one 0.3 and one 1.0 mg PFOA/kg BW intact mouse. These results in KO mice demonstrate that PFOA has potential hepatotoxic and hepatocarcinogenic effects independent of PPAR-α signaling. This abstract does not necessarily reflect NIEHS or EPA policy.

PS 1090 INVOLVEMENT OF CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) IN THE PROCESS OF LIVER HYPERTROPHY AND HEPATOCARCINOGENESIS INDUCED BY CYP2B-INDUCING NON-GENOTOXIC HEPATOCARCINOGENS IN MICE (1).

Y. Sakamoto[1], M. Yoshida[1], K. Inoue[1], M. Takahashi[1], Y. Taketa[1], S. Hayashi[1], S. Ozawa[2] and A. Nishikawa[1]. *[1]Division of Pathology, NIHS, Setagaya, Tokyo, Japan and [2]School of Pharmacology, Iwate Medical University, Morioka, Iwate, Japan.*

CAR is a nuclear receptor which is involved in metabolism of xenobiotics, regulating some metabolism related genes such as *Cyp2b*. CAR plays an essential role in the induction of liver hypertrophy and carcinogenesis in rodents treated with phenobarbital (PB). However, it is unclear whether liver hypertrophy and/or carcinogenesis pathways in other CYP2B inducers are similar to those in PB or not. In this study, we aimed to clarify the involvement of CAR in chemical-inducible liver hypertrophy using C3H-derived CAR knockout (CARKO) mice. Piperonyl butoxide (PBO) and decabromodiphenyl ether (DBDE) were selected as CYP2B-inducing, non-genotoxic hepatocarcinogens in rodents and compared with PB. Six-week-old male CARKO and wild mice were treated with PBO, DBDE or PB for 4 weeks at 5000, 50000 or 500 ppm in diet, respectively. Consequently, increases in liver weight and severe centrilobular/diffuse hepatocellular hypertrophy were induced by all chemicals in wild mice. In CARKO mice, liver hypertrophy was observed only with PBO, but not with DBDE or PB. In immunohistochemistry, the hypertrophied hepatocytes in wild mice were strongly positive for CYP2B in all treated groups. The signals were faint in CARKO mice with DBDE or PB, while being slightly and diffusely positive with PBO. *Cyp2b10* mRNA expression was drastically increased in all treated groups of wild mice and moderately in PBO group of CARKO mice. PBO in both genotypes significantly increased *Cyp3a11* mRNA expression. Our results indicate that CAR is essential for liver hypertrophy induced by DBDE or PB, while PBO induces liver hypertrophy through CAR-independent pathway. CARKO mouse is a useful tool to detect whether chemical induced liver hypertrophy is mediated by CAR or not.

PS 1091 EXPOSURE TO TCDD INCREASES HEPATIC STELLATE CELL PROLIFERATION AND ACTIVATION.

W. A. Harvey, C. J. Doolittle and K. A. Mitchell. *Biology, Boise State University, Meridian, ID.*

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a halogenated aromatic hydrocarbon that elicits toxicity by activating the aryl hydrocarbon receptor. One of the target organs for TCDD toxicity is the liver. We have previously shown that exposure to TCDD suppresses hepatocyte proliferation during liver regeneration induced by 70% partial hepatectomy (PH). In the present study, we investigated the possibility that TCDD alters the activation of hepatic stellate cells (HSCs), which are non-parenchymal cells that regulate liver regeneration through the production of cytokines and growth factors. Mice were treated with TCDD (20 μg/kg) or peanut oil control 24 hr prior to PH, and plasma and liver tissue were collected 0-72 hr later. Exposure to TCDD increased expression of the HSC activation marker, alpha-smooth muscle actin, in the regenerating liver 48 hr post-PH, and increased plasma levels of monocyte chemoattractant protein (MCP)-1, which is a chemokine that is abundantly produced by HSCs. *In vitro* studies with the LX-2 human hepatic stellate cell line revealed that TCDD treatment increased cell proliferation by at least 25% and increased MCP-1 production in supernatants almost two-fold over controls. These results indicate that HSCs may be targeted by TCDD both *in vivo* and *in vitro*. Hence, it is possible that inappropriate HSC activation may contribute to the suppression of liver regeneration observed in TCDD-treated mice through the production of soluble mediators, such as MCP-1, that enhance inflammation or suppress hepatocyte proliferation.

PS 1092 DETERMINATION OF *IN VIVO* KUPFFER CELL FUNCTIONS BY CONTRAST ENHANCED ULTRASOUND (CEUS) IMAGING.

V. Joaquin[1], T. Tomoo[1], E. Skuba[5], T. Wang[3], V. Richard[4], S. Chibout[2], F. Pognan[2], N. Shangari[1] and A. Wolf[2]. *[1]iTOX, Preclinical Safety (PCS), Novartis Institutes of Biomedical Research (NIBR), East Hanover, NJ, [2]iTOX, PCS, NIBR, Basel, Switzerland, [3]PCS, NIBR, Emmeryville, CA, [4]iTOX, PCS, NIBR, Cambridge, MA and [5]PCS, NIBR, East Hanover, NJ.*

Contrast enhanced ultrasound (CEUS) imaging utilizes intravenously injected gas-filled microbubbles to increase the echogenicity within the vasculature, resulting in an image with increased contrast where there is blood flow. Activated macrophages have been shown to readily engulf these microbubbles. We used CEUS to show a significant reduction in liver parenchymal contrast enhancement two days after depletion of the Kupffer cell population in rats using a single 10 mg/kg dose of gadolinium chloride. Immunohistological analysis confirmed the loss of macrophages within the liver. Macrophage depletion lead to a prolongation of microbubbles in circulation for up to 15 minutes longer than in saline treated rats. This technique was then used to observe the effect of XYZ123 (CSF1R inhibitor) treatment on the Kupffer cell population. Our objective was to observe the change in male rats using CEUS of the liver parenchyma and vasculature during 7 days of oral dosing with either 10 mg/kg or 150 mg/kg of XYZ123 plus 1-week of recovery period. Results from day 2, 5, and 8 of XYZ123 dosing showed no changes in liver parenchymal CEUS imaging. Similarly, no changes were observed during the recovery period. However, the vascular circulation revealed increased CEUS imaging signals compared to controls suggesting a delayed uptake of the microbubbles from the vasculature in a time- and dose-dependent manner during the dosing period. All groups returned to control contrast enhancement during the recovery phase. In conclusion, we show that although there was no change in CEUS imaging of the liver parenchyma following XYZ123 treatment, there may be an effect on the functions of a non-hepatic population of macrophages reflected by the prolonged duration of vascular CEUS imaging.

PS 1093 TCDD ENHANCES THE RESPONSE OF INNATE IMMUNE CELLS TO CONCANAVALIN A ADMINISTRATION.

A. M. Fullerton, R. A. Roth and P. E. Ganey. *Department Pharmacology & Toxicology, Center for Integrative Toxicology, Michigan State University, East Lansing, MI.*

Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a toxic and persistent environmental pollutant, has been linked to a number of adverse health conditions. In addition, inflammation has been found to be a major contributor to the pathology of a number of physiological disorders and diseases, including the development of liver injury. Using a model of inflammatory liver injury induced by concanavalin A (con A), we demonstrated that mice treated with TCDD and con A had greater liver injury compared to either treatment alone. In addition, mice treated with TCDD and con A had altered plasma concentrations of inflammatory cytokines, including increased interferon gamma (IFNγ). Since innate immune cells in the liver, particularly NK and NKT cells, produce IFNγ upon activation, we tested the hypothesis that TCDD pretreatment enhances the activation of innate immune cells after con A administration. Mice were treated with 30 μg/kg TCDD or vehicle, and 4 days later they were given 6 mg/kg con A or vehicle. Hepatic leukocytes were isolated, and flow cytometry was used to determine the activation and accumulation of various innate immune cells. NKT cells from TCDD/con A-treated animals showed increased expression of the early lymphocyte activation marker

CD69 compared to all other treatment groups. In addition, Fas ligand expression was increased in NK and NKT cells from cotreated mice, suggesting increased cytolytic activity of these cells, a possible contributor to liver injury. Compared to other treatment groups, livers from TCDD/con A-cotreated mice showed increased accumulation of both Gr-1-positive (granulocytes) and F4/80-positive (monocytes) cells as early as four hours after Con A treatment. The results suggest that TCDD exposure increased the recruitment and activation of innate immune cells in the liver in response to the inflammatory stimulus con A. This effect could contribute to the enhancement by TCDD of con A-induced liver injury. (Supported by NIH grant ES04911 and T32 ES007255.)

PS 1094 A MICROPATTERNED HUMAN HEPATOCYTE CO-CULTURE MODEL ALLOWS DETERMINATION OF TOXICITY AT MORE RELEVANT CONCENTRATIONS THAN HEPATOCYTE SANDWICH CULTURES.

D. Keller[1], S. Krzyzewski[2] and S. Khetani[2]. [1]*Preclinical Safety, sanofi-aventis U.S., Malvern, PA and* [2]*Hepregen Corp., Medford, MA.*

Use of human hepatocytes in sandwich cultures for determining toxicity potential is limited by the short functional lifespan of hepatocytes (~5 days). HepatoPac is a system of micropatterned human hepatocytes co-cultured with mouse embryonic 3T3-J2 fibroblasts enabling functional survival of hepatocytes for >30 days. This study was performed to determine if HepatoPac would allow for use of lower, more relevant concentrations in toxicity studies than used with sandwich cultures. Hepatocytes from 2 human donors were cultured in the HepatoPac system for up to 3 weeks or in sandwich cultures for 4 days and exposed to multiples of the human Cmax of pravastatin (PS), simvastatin (SS) or cerivastatin (CS). ATP levels, albumin synthesis and urea secretion were used to determine the viability and function of hepatocytes at various time points. Donor 1 results: In HepatoPac a slight decrease in ATP was seen with CS at 50x Cmax on day 3. Concentration-related decreases in ATP, albumin synthesis and urea secretion were seen with CS on days 7 and 15, beginning at 25x Cmax. No significant changes were observed with PS, and with SS only albumin synthesis was affected at 50x Cmax on day 15. In sandwich cultures the only change observed was decreased albumin with PS and CS at 50x Cmax. Donor 2 results: In HepatoPac, concentration-related ATP depletion and decreased urea secretion were seen with CS on days 8 and 15 from 10x Cmax, while albumin synthesis was affected at ≥25x Cmax. The changes seen with Donor 2 were of a smaller magnitude than for Donor 1. Only urea was affected on day 15 with SS, and no changes were seen with PS. With sandwich cultures, very slight (<10%) decreases in ATP were seen at ≥50x Cmax for PS and at ≥10x Cmax for CS, with no changes in albumin or urea. Overall, the results show that extended functional life of hepatocytes may allow for determination of toxicity at more therapeutically-relevant concentrations than possible with sandwich cultures, and that there is donor variability.

PS 1095 DEVELOPMENT OF AN *IN VITRO* HEPATOCYTE MODEL TO INVESTIGATE CHEMICAL MODE OF ACTION.

S. D. Hester[1], J. Harrill[1], C. Corton[1] and L. Murphy[2]. [1]*Systems Biology Branch, U.S. EPA, Durham, NC and* [2]*Carcinogenesis, U.S. EPA, Durham, NC.*

There is a clear need to identify and characterize liver in vitro models that can be used to replace animals for mode of action (MOA) analysis. Our goal is to use in vitro models for MOA prediction which recapitulate critical cellular processes underlying in vivo hepatotoxicity and which express key mediators of hepatotoxicant MOA, including nuclear receptors. We assessed the potential of an immortalized mouse liver cell line, AML-12, as an in vitro model for detecting chemical-mediated increases in cell proliferation. Cultures were exposed to several reference compounds known to increase hepatocyte proliferation in vivo and which activate proliferation through different MOA, including: tumor necrosis factor-alpha (TNF-alpha, WY-14,643, epidermal growth factor (EGF), the fungicide propiconazole and phenobarbital. Cell proliferation was assessed by incorporation of BrdU at 4, 24, and 48 hrs using the Cellomics ArrayScan VTI high content image analysis platform. BrdU incorporation increased after phenobarbital (200, 500, and 1000 uM), WY-14,643 (25, 50 uM), EGF (5 uM) and propiconazole (10, 25, and 50 uM) exposure. In addition, more than 50 other cellular measurements were captured including number of nuclei, nuclear area, cell count per field, BrdU % positive, average intensity of BrdU positive cells on a well to well basis. Future assessments of this in vitro mouse model will include markers for cytotoxicity and apoptotic endpoints. The coupling of phenotypic data with expression of biomarker gene targets of nuclear receptors will allow a prediction of MOA for chemicals of interest to EPA. This abstract does not reflect EPA policy.

PS 1096 QUANTITATIVE (Q)PCR ANALYSIS OF RNA EXTRACTED FROM FORMALIN-FIXED PARAFFIN EMBEDDED (FFPE) LIVER FROM AFLATOXIN B1 (AFB1) TREATED RATS: CORRELATION WITH MICROARRAY DATA.

B. A. Merrick, S. S. Auerbach, P. S. Stockton, J. F. Foley, D. E. Malarkey, R. C. Sills, R. D. Irwin and R. R. Tice. *National Toxicology Program, NIEHS/NIH, Research Triangle Park, NC.*

AFB1, a genotoxic carcinogen, induces multiple hepatocellular carcinomas in chronically-treated rats. Previous microarray analysis in liver showed that a 90-day exposure of male F344 rats to 1 ppm AFB1 in feed altered the expression of hundreds of genes prior to liver tumor development or detectable histopathological changes. In this study, we evaluated the expression of 11 genes (selection based on microarray data) by (q)PCR using RNA extracted from FFPE liver prepared from the same rats (control and treated, 6 per group) used in the microarray study. RNA extraction from four combined sections/archival liver paraffin block yielded 40 to 50 μg RNA that ranged in size from 0.1 to 4 kB, measured by Bioanalyzer. qPCR showed increased fold expression in AFB1 treated rats compared to controls of 91X for the oncogene, Mybl2; 32X and 23X, respectively, for the drug transporters, Abcb1b and Abcc3; 91X for the disintegrin-metalloprotease, Adam8; 190X for DNA damage-inducible, Ddit4L; 54X for the glutamate receptor subunit, Grin2c; 11X for xenobiotic-responsive, uncharacterized protein c8orf46; and 65X for the cadherin, Cdh13. For fragile site genes, Fhit expression was lower by 1.5X than control and Wwox was unchanged. Expression of the chemokine, Cinc1, was unchanged. Overall, expression data for select genes from qPCR and microarray platforms agreed well for directionality and fold change. By qPCR, 5 genes exhibited higher expression responses, 4 genes exhibited similar responses, and 1 gene showed a lower response compared to microarray. These data suggest that RNA isolated from FFPE tissues is of sufficient quality for routine qPCR analysis.

PS 1097 SIMILARITIES AND DIFFERENCES IN THE EXPRESSION OF DRUG METABOLIZING ENZYMES BETWEEN HUMAN HEPATIC CELL LINES AND PRIMARY HUMAN HEPATOCYTES.

L. Guo, S. Dial, L. Shi, W. Branham, J. Fang, B. Green, J. Kaput and B. Ning. *NCTR, Jefferson, AR.*

In addition to primary human hepatocytes, hepatoma cell lines and transfected non-hepatoma hepatic cell lines have been used for pharmacological and toxicological studies. However, a systematic evaluation and a general report of the gene expression spectra of drug metabolizing enzymes and transporters (DMETs) in these in vitro systems are not currently available. To fill this information gap and to provide reference for future studies, we systematically characterized the basal gene expression profiles of 251 drug metabolizing enzymes in untreated primary human hepatocytes from 6 donors, 4 commonly used hepatoma cell lines (HepG2, Huh7, SK-Hep-1 and Hep3B), and one transfected human liver epithelial (THLE2) cell line. A large variation in DMET expression spectra was observed between hepatic cell lines and primary hepatocytes, with the complete absence or much lower abundances of certain DMETs in hepatic cell lines. Furthermore, the basal DMET expression spectra of 5 hepatic cell lines are summarized, providing references for researchers to choose carefully appropriate in vitro models for their studies of drug metabolism and toxicity, especially for studies with drugs in which toxicities are mediated through the formation of reactive metabolites.

PS 1098 ENHANCEMENT OF NATURALLY OCCURRING BASOPHILIC FOCI IN THE LIVERS OF HAN WISTAR RATS TREATED WITH AZD9056.

P. A. Duffy, J. G. Evans, P. J. Kerry, M. O'Donovan, M. Coulson and H. Powell. *Global Safety Assessment, AstraZeneca, Macclesfield, Cheshire, United Kingdom.*

AZD9056 is a P2X7 antagonist previously under development for the treatment of inflammatory conditions such as rheumatoid arthritis. In support of large scale clinical trials AZD9056 was evaluated in a series of non-clinical rodent and non rodent toxicity studies of up to 6 months duration. Histopathological examination of the livers from rats dosed orally with AZD9056 for six months revealed an increase in the incidence of basophilic foci in treated animals compared to controls. The effect was observed at doses of 75 and 125 mg/kg/day in females only and was persistent in recovery animals after a period of 28 days. As part of the assessment of the consequences for clinical risk, further investigations were undertaken to evaluate the nature of the lesion and the possibility of genotoxicity related proliferative

events in the liver. AZD9056 was evaluated in vitro in the Ames test and the mouse lymphoma assay and in vivo in a rat micronucleus study and UDS study. There was no evidence of genotoxicity seen in any of these studies. In further studies rats dosed with AZD9056 were evaluated for cellular proliferation in hepatic foci by co administration of BrdU via osmotic minipumps. A marginal increase in hepatocyte proliferation, as determined by BrdU incorporation was detected in animals dosed at 125 mg/kg/day. There were no effects on hepatocyte cell proliferation at doses up to 75 mg/kg/day. Foci of altered hepatocyte morphology and histopathological staining are a naturally occurring age related finding in the rat. AZD9056 at doses of 75 mg/kg/day for 6 months exacerbates the formation of these foci in a dose dependent manner. There is no evidence of a genotoxic change that may be related to these findings and cellular proliferation has a clear no effect level. It was concluded that these findings have no impact on clinical safety of AZD9056.

PS 1099 THE EFFECT OF VITAMIN E DEFICIENCY ON DCA- AND TCA-INDUCED OXIDATIVE STRESS IN THE LIVERS OF MALE B6C3F1 MICE FOLLOWING SUBCHRONIC EXPOSURE.

J. Cearfoss and E. Hassoun. *Pharmacology, University of Toledo, Toledo, OH.*

Dichloroacetate (DCA) and Trichloroacetate (TCA) are water disinfection by products that are known to induce hepatotoxicity/ hepatocarcinogenicity in rodents. Previous studies in our lab have indicated induction of various biomarkers of oxidative stress (OS) in the livers of mice after subchronic exposure. To assess the effect of marginal Vitamin E deficiency on TCA- and DCA-induced OS, two groups of male B6C3F1 mice were fed either normal diet supplemented with vitamin E (E-normal group), or diet that was not supplemented with the vitamin(E-deficient). The two groups were subdivided into subgroups that were treated subchronically, by gavage, with the following: water (control), 77 mg DCA/kg/day, or 77 mg TCA/kg/day. Mice were sacrificed and the livers were collected and assayed for biomarkers of OS, such as production of superoxide anion (SA), lipid peroxidation (LP) and DNA single strand breaks (SSBs), as well as for antioxidant enzyme activities, including the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX). DCA and TCA produced significant increases in the production of SA, LP and DNA-SSBs in the two groups when compared with the corresponding controls of each groups. However, the increases in those biomarkers were less significant in the E-deficient group when compared with the E-normal group. While DCA and TCA treatment resulted in SOD and GSH-Px inhibition in the E-normal group, they induced those enzyme activities in the E-deficient group. CAT activity on the other hand was not significantly changed when comparing various treatments in the E-deficient with the E-normal group. These results suggest that marginal vitamin E deficiency in mice results in compensatory increases in antioxidant enzyme activities to provide partial protection against DCA- and TCA-induced OS. (Supported by NIH/NIEHS grant # R15ES013706-01A2)

PS 1100 O- AND N-DEMETHYLATION OF LEVOMETHORPHAN BY HUMAN CYTOCHROME P450 ENZYMES.

M. Sunouchi[1], A. Miyajima-Tabata[2], R. Kikura-Hanajiri[3] and Y. Goda[3]. [1]*Division of Pharmacology, National Institute of Health Sciences, Tokyo 158-8501, Japan,* [2]*Division of Medical Devices, National Institute of Health Sciences, Tokyo 158-8501, Japan and* [3]*Division of Pharmacognosy and Phytochemistry, National Institute of Health Sciences, Tokyo 158-8501, Japan.* Sponsor: A. Hirose.

Levomethorphan is the enantiomer of dextromethorphan. Dextromethorphan is an antitussive medicine and its metabolism has been reported well, whereas levomethorphan is an opioid analgesic with few metabolism studies. We have established the chiral analytical method for dextromethorphan, levomethorphan and their metabolites in biological samples using LC-MS/MS. In this study, we investigated the metabolic properties of levomethorphan by human and rat drug metabolizing enzymes and compared with the metabolism of dextromethorphan.
These drugs were incubated with rat or pooled human liver microsomes in NADPH generating system. Recombinant cytochrome P450 (CYP) 2D6 and CYP3A4 were also used for the particular analyses of the enantioselective metabolisms. The separation of these drugs and their metabolites was achieved on a Chiral CD-Ph column in a 0.1% formic acid-acetonitrile by a linear gradient program. MRM was used in the positive mode of an ESI-MS/MS for the quantitative analysis. After 20-min incubation with liver microsomes, levomethorphan was metabolized to O-demethyl and N-demethyl form by 63% and 15% (rat), 11% and 7.1% (human), respectively. The total amount of these metabolites from levomethorphan was higher than that from dextromethorphan both in rat (2.0 times) and human (3.1 times) microsomes. In the case of the incubation with CYP2D6, O-demethylation was the major metabolism pathway from levomethorphan as well as from dextromethorphan. O-demethylation of levomethorphan by CYP2D6 showed 2.3 times higher than dextromethorphan. In the case of CYP3A4, only N-demethyled metabolites of these drugs were detected and N-demethylation of these drugs was showed almost similar degree. These results suggest that the enantioselective metabolism of levomethorphan is mainly related to the O-demethylation by CYP2D6.

PS 1101 EFFECT OF ACARBOSE ON ALANINE AMINOTRANSFERASE 1 AND 2 PROTEINS AND GLUCONEOGENESIS IN RAT TISSUES.

K. Kumagai, S. Arakawa, T. Matsuyama, K. Kai, M. Teranishi and A. Sanbuissho. *Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd., Shizuoka, Japan.*

Acarbose, an inhibitor of α-glucosidase and α-amylase, causes the elevation of serum alanine aminotransferase (ALT) activity without any histopathological changes in rats. The mechanism for serum ALT elevation is still unclear, although it is assumed to be due to increased gluconeogenesis by the pharmacological effect of acarbose. Rat ALT isozymes, ALT1 and ALT2, have been recently identified and have been reported that the two isozymes are expressed differently in tissues. The present study investigated the effect of acarbose on rat ALT1 and ALT2 proteins in the serum and in the isozyme-expressed tissues: liver, heart, skeletal muscle, jejunum, and white and brown adipose tissues. The effect of acarbose on gluconeogenesis in the liver was also evaluated. Acarbose dissolved in distilled water was administered by gavage to male F344 rats at dose levels of 0 or 2000 mg/kg/day for 7 days. The acarbose-treated rats had a significant increase in serum ALT activity compared with the control. Acarbose also induced increased serum levels in aspartate transferase and non-esterified fatty acid, and decreased serum levels in triacylglycerol and glucose. No histopathological changes by the acarbose treatment were observed in the liver, heart, skeletal muscle, jejunum, white or brown adipose tissues. Western blot analysis revealed that ALT1 protein levels in the serum and liver of acarbose-treated rats were significantly higher than those in the control. On the other hand, compared to the control, acarbose-administered rats had no increases in ALT1 proteins in other tissues or ALT2 proteins in any tissues. Moreover, serum ALT2 protein was not detected in either control or acarbose-administered rats. Phosphoenolpyruvate carboxykinase protein in the acarbose-treated rat livers was significantly increased compared to the control. In conclusion, the results of the present study suggest that acarbose causes increases in gluconeogenesis and ALT1 protein in the liver, resulting in the elevation of ALT1 protein and ALT activity in the serum.

PS 1102 ENZYMES AND OXIDATIVE DAMAGE INDICATORS IN RATS FOLLOWING SUBACUTE OR SUBCHRONIC MANGANESE CHLORIDE EXPOSURE.

P. Huang[1], G. Li[2] and Z. Sun[1]. [1]*Department of Toxicology and Sanitary Chemistry, School of Public Health and Family Medicine, Capital Medical University, Beijing, China and* [2]*Beijing Research Center for Preventive Medicine, Beijing, China.* Sponsor: W. Zheng.

Manganese (Mn) toxicity is most often found in mining and welding industry workers. Accumulation of manganese in the brain can result in a syndrome similar to that of Parkinson's disease. Observations on former Mn-alloy workers suggested that residual effects could last for years after exposure. The objective of this study was to assess effects of Mn in the liver of rats following subacute or subchronic exposure and after recovery. Male Sprague-Dawley rats weighing 110 to 120 g were exposed to 6.0 mg Mn/kg b.w. using MnCl2 for 30 days, 90 days, or for 90 days followed by a 30-day post-exposure recovery period. Results showed that serum AST and ALT increased compared to controls. Mn concentrations in the liver of treated animals increased 1.5 to 2.5-fold compared to controls. Similarly, concentrations of Ca and Cu increased in treated animals, while concentrations of Fe, Mg and Zn decreased in liver tissues after subacute or subchronic exposure. Subacute or subchronic Mn exposure also inhibited GPx activity, decreased GSH levels and increased MDA levels in liver tissues. All of the indicators returned to normal after the recovery period. We further evaluated the effects of manganese on rat hepatocyte mitochondria, membranes, and nuclei in the three periods. The results showed significant inhibition of mitochondrial SDH, cellular Na+,K+-ATPase, Ca2+,Mg2+-ATPase, Cu2+-ATPase, and GPx activities in hepatocytes. In addition, GSH levels decreased while MDA levels increased in hepatocyte mitochondria, membranes, and nuclei. All of these markers were normal after the recovery period. Taken together, these data suggest that MnCl2 exposure injured rat liver tissue, that the extent of injury is positively correlated with exposure time, and that the effect on mitochondria is presumably more serious than that on nuclei and membranes. Most biomarker alterations returned to pre-exposure levels by themselves after stopping Mn exposure.

PS 1103 THE ROLE OF 3, 3', 4, 4', 5-PENTACHLOROBIPHENYL (PCB 126) IN COPPER DISPOSITION IN RODENT LIVER.

M. Li[1, 2], I. Lai[1, 2], B. Wels[3], D. Simmons[3], G. Ludewig[1, 2] and L. Robertson[1, 2]. [1]*Interdisciplinary Program in Human Toxicology, University of Iowa, Iowa City, IA,* [2]*Occupation and Environmental Health, University of Iowa, Iowa City, IA and* [3]*State Hygienic Laboratory, University of Iowa, Iowa City, IA.*

Copper, an essential trace element and ubiquitous in animals and humans, is essential for the proper functioning of the electron transport chain in the mitochondria as well as an antioxidant in copper-zinc superoxide dismutase (CuZnSOD). However, in its free form, copper can also participate in Fenton-like reactions that result in the production of reactive hydroxyl radicals. In previous studies copper levels in rodent liver were increased following exposure to aryl-hydrocarbon receptor agonists, including the most potent polychlorinated biphenyl (PCB) congener, PCB 126. Increased hepatic copper was accompanied by several histological changes, including steatosis. In an effort to assess the effect of dietary copper on these changes, male Sprague-Dawley rats were fed an AIN-93G diet with one of three dietary copper levels: low (2 ppm), adequate (6 ppm), and high (10 ppm). After three weeks, rats from each dietary group were given a single ip injection of corn oil (control), 1, or 5 μmol/kg body weight PCB126 in corn oil, followed two weeks later by euthanization. Growth rate was slowed by PCB 126 in a dose-dependent manner, significantly at the highest dose (40-75%). Relative liver weight was increased in a dose-dependent manner (30-65%) by PCB 126, while there was no effect on growth or liver weight by dietary copper. Hepatic cytochrome P450 activity was maximally-induced by 1 μmol/kg PCB 126. Increasing dietary copper and increasing dose of PCB 126 both increased hepatic copper levels. Blood copper and serum ceruloplasmin levels were biphasic, diminishing with increasing PCB 126 dose at low dietary copper, while increasing in rats fed high dietary copper. Hepatic CuZnSOD activity was not significantly affected by dietary copper. We conclude that while reducing dietary copper reduced hepatic copper levels, the function of essential copper enzymes were not negatively affected. (Supported by NIEHS P42 ES 013661)

PS 1104 ENTEROHEPATIC CIRCADIAN RHYTHM IN MICE: CONTRIBUTIONS OF BILE ACIDS.

Y. J. Zhang, G. L. Guo and C. D. Klaassen. *Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Diurnal fluctuation of bile acid (BA) concentration in the enterohepatic system of mammals has been known for a long time. Recently, BAs have been shown as signaling molecules beyond their well-established roles in dietary lipid absorption and cholesterol homeostasis. The current study depicts diurnal variation of individual BAs detected by ultra-performance liquid chromatography/mass spectrometry (UPLC/MS) in serum and livers collected from C57BL/6 mice fed regular chow or chow containing cholestyramine (resin). Circadian rhythms of mRNA of vital BA-related nuclear receptors, enzymes, and transporters in livers and ilea were determined in control- and resin-fed mice, as well as in farnesoid X receptor (FXR) null mice. The circadian profiles of BAs show enhanced bacterial dehydroxylation during the fasting phase and efficient hepatic reconjugation of BAs in the fed phase. The resin removes more than 90% of BAs with β-hydroxy groups, such as muricholic acids and ursodeoxycholic acid, from serum and livers, but markedly increases deoxycholic acid and lithocholic acid in both compartments. Data from both resin-fed and FXR-null mouse models indicate that BAs regulate their own biosynthesis through the FXR-regulated ileal fibroblast growth factor 15. BA flux also influences the daily mRNA levels of multiple BA transporters. In conclusion: BA concentration and composition exhibit circadian variations in mouse liver and serum, which influences the circadian rhythms of BA metabolizing genes in liver and ileum. The diurnal variations of BAs appear to serve as a signal that coordinates daily nutrient metabolism in mammals. This study is supported by NIH grants DK-081461, ES-009716, ES-009649, ES-013714, and RR-021940.

PS 1105 MYELOPEROXIDASE-DEPENDENT METABOLISM OF P-CRESOL: A NOVEL PATHWAY FOR XENOBIOTIC AND ENDOBIOTIC BIOTRANSFORMATION DURING INFLAMMATION.

J. Houghton[1], K. Hayakawa[1], D. DeGroot[2], B. Zhao[2], M. Denison[2] and J. P. Eiserich[1]. [1]*Department of Internal Medicine, University of California, Davis, Davis, CA and* [2]*Department of Environmental Toxicology, University of California, Davis, Davis, CA.*

Myeloperoxidase (MPO) is a heme protein abundantly expressed in neutrophils that is traditionally regarded as a critical host defense enzyme that facilitates bacterial killing. Based upon its oxidizing P450-like behavior, we speculated that MPO could play an unexpected role in xenobiotic and endobiotic metabolism. p-Cresol (4-methylphenol) is a natural product that humans are exposed to both exogenously (ie. cigarette smoke) and endogenously (ie. tyrosine metabolism by gut microflora). Herein we demonstrate that MPO is capable of converting p-cresol into nitrated and oxidized metabolites, the latter with bioactivity not shared with the parent compound. Enzymatic experiments with MPO revealed that p-cresol was converted primarily to 4α,9β-dihydro-8,9β-dimethyl-3(4H)-dibenzofuranone, otherwise known as "Pummerer's ketone" (PK), with smaller yields of 2-nitro-4-methylphenol, and 2,2'-dihydroxy-5,5'-dimethyldiphenyl but no chlorinated products. In vitro and cell culture approaches revealed that PK is a ligand for the AhR and activates its ability to bind to the dioxin-response element (DRE), and induces a 5-fold induction in the expression of cytochrome P450 1A1 mRNA. Additionally, PK induces the activation and nuclear translocation of the transcription factor Nrf-2, and consequently induces the expression of heme oxygenase-1 (HO-1) and glutathione cysteine ligase catalytic (GCLC) subunit. Chemical reactions, coupled with LC/MS analyses, revealed that PK can react with the model thiol compounds glutathione (GSH) and N-acetylcysteine (NAC) to form covalent Michael addition products, suggesting that the cellular bioactivity of PK may be dictated by the formation of adducts with cysteine residues in critical proteins essential to transcriptional responses. Our data illustrate that MPO may play a previously unrecognized and important function in xenobiotic and endobiotic metabolism during inflammation.

PS 1106 TOXICITY, TOXICOGENOMIC, AND METABOLOMIC STUDY OF PENTAMETHYLCHROMANOL.

T. Parman[1], D. Bunin[1], H. H. Ng[1], T. Harrison[1], J. McDunn[2], I. M. Kapetanovic[3] and C. E. Green[1]. [1]*Biosciences, SRI International, Menlo Park, CA,* [2]*Metabolon, Inc., Durham, NC and* [3]*National Cancer Institute, Bethesda, MD.*

Pentamethyl-6-chromanol (PMCol) is a candidate chemopreventive agent against androgen dependent cancers. PMCol has been shown to cause hepatotoxicity, nephrotoxicity and hematological effects. The objectives of this study were to determine the mechanisms of toxicity and identify sensitive early markers of hepatic and renal injury using renal biomarkers (albumin, TIM-1, lipocalin-2, osteopontin, clusterin), toxicogenomics and metabolomic approaches. PMCol was administered to male Sprague-Dawley rats, 200 and 2000 mg/kg daily for 7 or 28 days. Changes in clinical chemistry included elevated alanine aminotransferase, total bilirubin, cholesterol and triglycerides, indicative of liver toxicity that was confirmed by microscopic findings (periportal hepatocellular hydropic degeneration and cytomegaly) in treated rats. Metabolomic evaluations of liver tissue revealed time and dose dependent changes including depletion of glutathione and glutathione conjugates, decreased methionine, and increased S-adenosylhomocysteine, cysteine, and cystine. PMCol treatment also decreased cofactor levels, e.g. FAD+ and NAD(P)+. Cholesterol and sphingomyelin were markedly higher in plasma from treated rats. Microarray analysis found that differentially expressed genes were enriched in the glutathione and cytochrome P450 pathways in liver by PMcol treatment. RT-qPCR of six upregulated genes (Akr7a3, Cyp1a1, Gpx2, Gsr, Gstp1, and Hmox1) and one down regulated gene (Acnat2) confirmed the microarray results. Metabolomic, urine biomarkers and histopathologic changes in kidneys were also observed but were minor compared to hepatic effects. In conclusion, metabolomics and toxicogenomics demonstrate that chronic exposure to high doses of PMCol induces liver damage and dysfunction, probably due to both inhibition of synthesis and depletion of liver glutathione, as well as modification of other drug metabolism pathways. (Supported by NCI Contract HHSSN261200433005C)

PS 1107 EVIDENCE OF PEPPERMINT OIL SKIN PENETRATION AND CYTOTOXICITY ON RAT HEPATOCYTES *IN VITRO.*

M. Dong[1], O. Weber[1], E. Di Lenarda[1], P. End[2], S. Chibout[1], A. Wolf[1] and F. Pognan[1]. [1]*Preclinical Safety, Novartis, Basel, Switzerland and* [2]*Drug Metabolism and Pharmacokinetics, Novartis, Basel, Switzerland.*

Peppermint oil is commonly used for digestive disorders taken orally and the relief of muscle pain by topical application. However, it has been suspected of potential adverse effects, including liver toxicity. The aim of the present study was to examine the acute effects of peppermint oil on rat hepatocytes in vitro.
Freshly isolated rat hepatocytes were cultured in collagen-sandwich configuration. Cells were treated daily with peppermint oil (China Oil) at the concentrations ranging from 0.001% to 0.3% (v/v) for up to three days. GC/MS analysis determined that this peppermint oil contained 1.18 % of pulegone and 0.78% of its main metabolite, mentofurane. Both have been described as hepatotoxicants in vivo in rat. Cellular ATP content, total glutathione, as well as ALT and AST release were determined.

The peppermint oil caused a concentration dependent increase in hepatocyte cytotoxicity. After one-day treatment, a significant, ~40%, reduction of cellular ATP and total glutathione content, along with ~10-fold increase in ALT/AST release were detected at concentration of 0.01% (v/v). No further increase in cytotoxicity was observed with time till the end of the treatment. Application of pulegone and mentofurane from the total oil on human cadaver skin showed that both significantly penetrated the dermis and the epidermis.
In conclusion, the present study demonstrated that toxic components of peppermint oil can cross the skin barrier and its in vitro toxicity in rat hepatocytes. Although the toxic concentrations were greater (~20 fold) than those recommended by authorities for human use, attention still needs to be taken to avoid potential liver damage resulting from overdosing.

PS 1108 MOLECULAR MECHANISM OF ALTERED EZETIMIBE DISPOSITION IN NON-ALCOHOLIC STEATOHEPATITIS.

R. N. Hardwick, C. D. Fisher, S. M. Street, M. J. Canet and N. J. Cherrington. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Ezetimibe (EZE) lowers serum lipid levels by blocking Neimann-Pick C1-Like 1-mediated cholesterol uptake in the intestine. Disposition of EZE and its pharmacologically active glucuronide metabolite (EZE-G) to the intestine is dependent on biliary efflux from the liver primarily via ABCC2. Because it has been suggested that ABCC2 and other hepatobiliary transporters are altered during non-alcoholic steatohepatitis (NASH), the purpose of the current study was to determine the effect of experimental NASH on EZE disposition in vivo. Rats were fed a methionine-choline deficient (MCD) diet for 8 weeks and then administered 10 mg/kg EZE either by intravenous bolus or oral gavage. Plasma and bile samples were collected over a 2 hour period followed by terminal urine, liver and intestinal tissue collection. EZE and EZE-G concentrations were determined by LC-MS/MS. Hepatic expression of the sinusoidal Abcc3 transporter was induced in MCD animals. This correlated with increased plasma concentrations of EZE-G in both i.v. and p.o. dosed MCD animals. Hepatic expression of the biliary Abcc2 and Abcb1 transporters was also increased in MCD; however, the biliary efflux of EZE-G was diminished in MCD animals. Interestingly, the cellular localization of Abcc2 appeared to be internalized away from the canalicular membrane in MCD liver. This phenomenon may account for the decreased efflux of EZE-G observed in MCD. Expression of the EZE metabolizing enzyme Ugt1a1 was not altered in MCD liver or intestine. Importantly, induction of ABCC2, 3 and ABCB1 protein was also found in human NASH liver samples compared to normal. Similarly, ABCC2 is also internalized in human NASH as evidenced by immunohistochemical staining and reduced glycosylation status of the protein. The combination of induced expression and altered localization of key efflux drug transporters in rodent and human NASH samples shifts the disposition profile of EZE toward plasma retention, thereby diminishing drug delivery to the active site.

PS 1109 DIETARY N-ACETYLCYSTEINE (NAC) REDUCES HEPATOCELLULAR LIPID ACCUMULATION FOLLOWING 3, 3', 4, 4', 5-PENTACHLOROBIPHENYL (PCB 126) EXPOSURE.

I. Lai[1, 2], A. Olivier[3], M. Li[1, 2], K. Dhakal[1, 2] and L. Robertson[1, 2]. [1]*Occupation and Environmental Health, University of Iowa, Iowa City, IA,* [2]*Interdisciplinary Program in Human Toxicology, University of Iowa, Iowa City, IA and* [3]*Department of Pathology, University of Iowa, Iowa City, IA.*

Glutathione (GSH) is a key intracellular antioxidant because of its thiol-containing cysteine group. However, due to difficulty in direct uptake, an alternative cysteine source, such as N-acetylcysteine, NAC, has been widely used as a thiol-donor to protect against reactive species. NAC as a supplement is of particular interest since previous studies have shown that exposure to aryl hydrocarbon agonists, including the most potent polychlorinated biphenyl (PCB) congener, PCB 126, caused liver pathology and reduced glutathione levels. We hypothesized that dietary NAC supplementation in rats will reduce PCB 126-induced toxicity. Male Sprague-Dawley rats were fed a standard AIN-93G diet or a modified diet supplemented with 1.0% NAC. After one week, rats from each dietary group were given a single ip injection of corn oil (control), 1, or 5 μmol/kg body weight PCB126 in corn oil, followed two weeks later by euthanization. Growth rate was slowed up to 20% by PCB 126 in a dose-dependent manner. Relative liver weight was increased in a dose-dependent manner (42-52%) by PCB 126, while NAC had no effect. Hepatic CYP1A1 activity was maximally-induced by PCB 126, indicating potent AhR activation. Total GSH levels in the liver were diminished in a dose-dependent manner (4-34%) by PCB 126, but not increased by NAC supplementation. Histological examination of liver tissue showed hepatocellular swelling and increased lipid accumulation (steatosis) caused by PCB 126. Interestingly, based on lipid quantification by osmium staining, hepatocellular lipid accumulation was diminished in rats supplemented with NAC. Based on these results, we conclude that NAC supplementation is a promising treatment in protecting against PCB 126-induced toxicity. (Supported by NIEHS P42 ES 013661)

PS 1110 SERUM CYTOKERATIN 18 AND CYTOKINE ELEVATIONS SUGGEST A HIGH PREVELENCE OF OCCUPATIONAL LIVER DISEASE IN ELASTOMER/POLYMER WORKERS HIGHLY-EXPOSED TO ACRYLONITRILE, BUTADIENE, AND STYRENE.

M. Cave[1, 2], K. Falkner[1], B. Costello[1], B. Gregory[1], L. Henry[1] and C. McClain[1, 2]. [1]*Department of Medicine/GI, University of Louisville, Louisville, KY and* [2]*Robley Rex Veterans Administration Medical Center, Louisville, KY.*

Objective: Occupational liver disease is likely to be under-recognized because, in many cases, routine serologic liver chemistries are not effective biomarkers. Cytokeratin 18 (CK18) is a novel serologic biomarker for occupational liver disease. We recently demonstrated that serum CK18 and pro-inflammatory cytokines were elevated in chemical workers with toxicant-associated steatohepatitis (TASH) due to high-level vinyl chloride exposures. The purpose of this study is to determine the prevalence of CK18 elevation in elastomer/polymer workers exposed to mixtures of acrylonitrile, 1,3 butadiene, and styrene. Methods: 82 chemical workers were evaluated. CK18 was determined by ELISA, and pro-inflammatory cytokines were measured by multi-analyte chemiluminescent detection. Results: Mean routine liver chemistries (aspartate aminotransferase, alanine aminotransferase, total bilirubin, albumin, and alkaline phosphatase) were in the normal range. In fact, only 3 of 82 total subjects had any single lab abnormality detected by these tests. However, 39% (32 of 82) had elevated CK18 levels which were not explained by alcohol or obesity, except potentially in 4 cases. The pattern of CK18 elevation was consistent with TASH in the majority of cases (78%). TNFα, IL-6, IL-8, MCP-1, and PAI-1 were increased in these workers compared to those with normal CK18 levels. Conclusions: These results suggest a high prevalence of occupational liver disease and TASH in elastomer/polymer workers with elevated CK18 and pro-inflammatory cytokines.
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PS 1111 MITOCHONDRIAL TOXICITY OF CHLOROACETALDEHYDE IN HEPG2 CELLS.

K. Falkner[1], B. Hill[1], B. Sansbury[1], J. Gaurdiola[1], C. McClain[2] and M. Cave[2]. [1]*Department of Medicine/GI, University of Louisville, Louisville, KY and* [2]*Louisville VA Medical Center, Louisville, KY.*

Background: We first described a high prevalence of toxicant-associated steatohepatitis in vinyl chloride workers. The steatohepatitis was atypical in that serum markers indicated a non-apoptotic cell death mechanism. Vinyl chloride toxicity is associated with its metabolites including chloroacetaldehyde (ClAc). We hypothesized that ClAc may disrupt mitochondrial function causing ATP depletion leading to necrotic cell death. Furthermore, nrf2 inducers may be protective against ClAc toxicity. Methods: HepG2 were grown in DMEM with 10% FBS in a 5% CO2 at 37OC. Cell death was determined by MTT assay. Protein sulfhydryl group modification was determined by the ability of cells to reduce BODIPY-IAM labeling. Respiration was determined using a Seahorse XF24 Extracellular Flux Analyzer. ATP levels were determined using a kit from Sigma. Results ClAc was toxic to HepG2 cells with an LD50 of 108.5 ± 2.1 uM and 89.7 ± 0.7 uM for serum-containing and serum free media, respectively. Treatment of cells for 1 hr with either 50 or 100 uM ClAc reduced the labeling of protein thiols by 24 and 41%, respectively. ClAc reduced oxygen consumption in a dose dependent manner by up to 69%, similar to that observed with 40 uM 4-hydroxynonenal. The ATP-dependent oxygen consumption was reduced by up to 86%. In HepG2 cells treated with ClAc for 4 h, ATP was depleted by 15% or 75% for treatments with 50 uM and 100 uM, respectively. Pretreatment with the nrf-2 activators, oltipraz and sulforaphane, decreased the toxicity of ClAc. Conclusions: ClAc is thiol reactive and capable of severely compromising mitochondrial respiration at concentrations in the toxic range. Near the LD 50, ATP depletion is significant and may result in necrotic cell death rather than ATP-dependent apoptosis. Nrf2 activators were protective against ClAc toxicity. NIH Funding Sources: P30ES014443-01A1, 1P01AA017103-01, K23AA18399-01A, R37AA010762, RC2AA019385, P20RR024489 and R01AA18869.

PS 1112 HGF ALTERED RESPONSE IN HIGH CHOLESTEROL FEED MICE HEPATOCYTES.

M. Dominguez, N. Nuño, D. Clavijo, C. Enriquez, V. Souza, L. Bucio, L. Gomez Quiroz and C. Gutierrez-Ruiz. *Health Sciences, Universidad Autónoma Metropolitana Iztapalapa, Mexico, Mexico.*

A high content of cholesterol in the diet can lead to over accumulation deposition in the liver where it can induce cellular damage,inducing membrane dynamics changes and improper signaling by receptors.Our objective was to characterize the oxidative damage induced by an atherogenic diet (2% cholesterol and 0.5 % sodium cholate) in the hepatocytes and the effect of the HGF as a protective cellular factor.CD1 mice were fed with an atherogenic diet (HC) for 48 h.Hepatocytes were isolated following the two-step collagenase perfusion method.Cells were treated or not with HGF (50 ng/ml.Catalasa, gamma-glutamyl-cysteine synthetase (γ-GCS),superoxide dismutase 1 (SOD1), phosphor-c-Met and total c-Met content were assayed by Western blotting;the wound-healing assay was performed at different times of incubation with HGF. Cholesterol was detected by filliping staining.Protein oxidation by oxyblot kit.Our data revealed that HC hepatocytes are under oxidative stress judged by protein oxidation and for the basal content in antioxidant enzymes such as catalasa,SOD1 and γ-GCS which were increased regarding chow hepatocytes.Although HGF treatment improve antioxidant enzymes and protein oxidation,the wound-healing test show that HGF failed in the induction of repair process in HC hepatocytes.In order to figure out the mechanism for this inappropriate response elicited by HGF we determined the phosphorylation of the HGF receptor c-Met.The result show that HGF-induced activation of c-Met in HC hepatocytes exhibit a delay (max activation 15 min) in comparison with chow hepatocytes (max activation 1 min,suggesting that cholesterol overload in the hepatocyte reduces the response of HGF/c-met.Our data show that hepatocytes with an overload in cholesterol content did not respond adequately to HGF treatment inducing an insufficient repair perhaps due to c-Met receptor is dependent of a correct functionality in lipids rafts where cholesterol plays imperative regulatory functions in signal transduction.

PS 1113 NRF2 ACTIVATION PROMOTES HYPERCHOLESTEROLEMIA AND GALLSTONE FORMATION.

M. A. Paranjpe[1], Q. Cheng[1], J. Moscovitz[1], D. Ajay[1], M. Yamamoto[2] and A. Slitt[1]. [1]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI and* [2]*Medical Biochemistry, Tohoku University, Sendai, Miyagi, Japan.*

Cholesterol (CH) gallstone disease is a common gastrointestinal disease and is associated with consumption of a CH rich diet. Some populations are more susceptible to gallstone formation, suggesting a genetic component to this susceptibility. The Kelch-like ECH-associated protein 1 (Keap1)-Nuclear factor-E2 related-factor 2 (Nrf2) transcriptional pathway mediates the antioxidant response, but emerging studies indicate a role for this pathway in modulating hepatic lipid homeostasis. Preliminary data demonstrate that Nrf2-null mice are resistant to CH gallstone formation when fed a lithogenic diet (15% fat, 1.25% CH, 0.5% sodium cholate) for nine weeks. The purpose of this study was to determine whether constitutive Nrf2 activation via Keap1-knock down causes increased CH gallstone formation. Adult female C57Bl/6 and Keap1-knockdown (Keap1-KD) mice were fed a standard or lithogenic diet for three weeks and gallbladders, livers and blood were collected. The lithogenic diet increased CH gallstones in Keap1-KD compared to C57Bl/6 mice. The lithogenic diet increased serum and liver CH levels in both the strains, but liver CH levels were significantly higher in Keap1-KD mice compared to C57Bl/6 mice. In addition, the lithogenic diet increased biliary cholesterol concentrations more in Keap1-KD mice compared to C57Bl/6 mice. Cyp7a1 mRNA expression in liver was decreased in Keap1-KD mice compared to C57Bl/6 after feeding a standard or lithogenic diet. Unlike C57Bl/6 mice, Keap1-KD mice failed to downregulate Hmg-CoA expression in liver after feeding the lithogenic diet. The lithogenic diet induced the expression of Abcg5, 8 and Abca1 mRNA levels to an equivalent degree in both the strains of mice. Overall, the data demonstrate that Nrf2 activation causes increased susceptibility to hypercholesterolemia, which is most likely due to differential modulation of CH synthesis and metabolism (NIH 5K22ES013782, 3R01ES016042-02S1).

PS 1114 NRF2 PROTECTS MOUSE LIVER FROM OXIDATIVE INJURY AND STEATOHEPATITIS INDUCED BY PERSISTENT ACTIVATION OF AHR.

H. Lu and C. D. Klaassen. *University of Kansas Medical Center, Kansas City, KS.*

Previous studies demonstrate that Nrf2, a master regulator of antioxidative responses, is essential in mediating the induction of certain antioxidative enzymes by acute activation of the aryl hydrocarbon receptor (AhR). However, the role of Nrf2 in the protection against oxidative stress and DNA damage induced by persistent activation of the AhR remains unknown, which was investigated in this study. Liver and blood samples were collected from wild-type (WT) and Nrf2-null mice 21 d after administration of a bolus low-toxic dose of TCDD 10 μg/kg (ip). After TCDD treatment, only Nrf2-null mice lost body weight; however, blood levels of ALT remained essentially unchanged in both genotypes, indicating a lack of extensive death of hepatocytes. Compared to livers of TCDD-treated WT mice, livers of TCDD-treated Nrf2-null mice had:1) swollen hepatocytes and markedly higher hepatic triglycerides; 2) similar induction of Cyp1a1/2, markedly higher expression of Cyp4a14, but lower catalase; 3) depletion of glutathione, elevation in lipid peroxidation, DNA damage, and apoptosis; 4) higher induction of proliferative genes mKi-67 and Top2a; 5) attenuated induction of Nqo1, Gsta2, and Ugt2b35, but higher induction of Ho-1, Prx-1, p21, and Fgf21; 6) compensatory induction of Gclc and mEH, 7) higher expression of fatty acid uptake and triglyceride synthetic genes; 8) higher expression of inflammatory and fibrotic genes; and 9) induction of c-jun and Gadd45β, a pro-survival gene induced by NF-KB. In conclusion, Nrf2 deficiency markedly increases oxidative stress, DNA damage, and steatohepatitis in livers with persistent activation of AhR. TCDD activation of AP-1 and NF-KB in Nrf2-null mice may cause compensatory induction of certain cytoprotective genes and prevent overt cell death; however, the markedly increased DNA damage and inflammation in TCDD-treated Nrf2-null mice indicates that Nrf2 deficiency may substantially increase the risk of liver carcinogenesis during persistent AhR activation (Supported by NIH grants ES-009716, ES-013714, ES-019487, DK081461, and RR021940).

PS 1115 TCDD EFFECTS ON HEPATIC LIPID COMPOSITION IN MICE.

M. M. Angrish[1, 2], B. D. Mets[2, 3], D. Wright[3] and T. R. Zacharewski[1, 2, 3]. [1]*Genetics Program, Michigan State University, East Lansing, MI,* [2]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [3]*Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI.*

TCDD and related compounds induce steatosis, hepatic vacuolization and hyperlipidemia in mice. In order to further investigate the effects on hepatic lipid composition, C57BL/6 female mice were treated with 30 μg/kg TCDD or sesame oil vehicle for 24, 72 or 168 h. TCDD elicited hepatic vacuolization and inflammation, and significantly increased relative liver weight (RLW) and hepatic triglyceride (TRG) levels (1.4-, 2.9- and 4-fold at 24, 72 and 168 h, respectively) compared to controls. GC-MS analysis of hepatic lipid extracts revealed a TCDD-dependent 2-fold increase in total hepatic fatty acids (TFAs) at 72 and 168 h post dose. More specifically, TCDD significantly induced long-chain (16C or greater) saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) levels at 168 h (161, 364 and 218%, respectively). However, there were significant decreases in the SFA/TFA ratio (25%), with increases in the MUFA/TFA ratio (167%), and no change in the PUFA/TFA ratio at 168 h. The reduction in PUFA/MUFA levels (60% at 168 h) was consistent with TCDD-induced lipid peroxide levels (LPO) (2.5-fold at 168 h). TCDD also induced the essential fatty acids 18:2n6 and 18:3n3 at all time points, with corresponding decreases (3-fold at 168 h) in product/precursor ratios (20:4n6/18:2n6 and 20:5n3/18:3n3). These changes can be partially explained by AhR-mediated induction of Scd1 and Elovl5 (2-4-fold at 24-72 h). Furthermore, computational analysis from -10,000 bp through the 5'UTR identified putative dioxin response elements (DRE) and ChIP-chip revealed AhR enriched regions within the Scd1 and Elovl5 loci. Collectively, TCDD-induced alterations in hepatic SFA, MUFA, PUFA and LPO levels are consistent with changes in RLW, vacuolization, inflammation and TRG levels. Gene expression, *in silico* DRE search and ChIP-chip studies suggest that these effects are AhR mediated. Funded by SBRP P42ES04911.

PS 1116 PHARMACOLOGIC NRF2 ACTIVATION PROMOTES HYPERCHOLESTEROLEMIA IN MICE.

J. Moscovitz, M. Paranjpe and A. Slitt. *University of Rhode Island, Kingston, RI.*

Hypercholesterolemia is associated with consumption of a CH rich diet. Some populations are more susceptible to hypercholesterolemia, and it is known that there is a hereditary component to susceptibility. The Kelch-like ECH-associated protein 1 (Keap1)-Nuclear factor-E2 related-factor 2 (Nrf2) transcriptional pathway mediates the antioxidant response, but emerging studies indicate a role for this pathway in modulating hepatic lipid homeostasis. The purpose of this study was to determine whether administration Nrf2 activators affects lithogenic feeding-induced hypercholesterolemia in mice. Male C57Bl/6 mice were pair-wise fed i) standard diet, ii) lithogenic diet (LD, 15% fat, 1.25% CH, 0.5% sodium cholate), iii) standard diet with butylated hydroxyl anisole (BHA, 0.1% w/w) or oleanolic acid (0.1% w/w), iv) LD with butylated hydroxyl anisole (0.1% w/w) or oleanolic acid (OA, 0.1% w/w) for six weeks. Serum, livers, and gallbladders were collected. BHA and

OA did not affect food consumption. BHA in standard chow significantly decreased body weight. The LD did not increase body weight alone, but significantly increased body weight when administered in combination with BHA or OA. BHA in standard chow increased liver weight, but OA did not. The LD significantly increased liver weight by 50%, with the BHA addition increasing liver weight an additional 50% more than the LD alone. Liver weight was increased to a similar degree in mice that received LD diet alone or in combination with OA compared to the standard diet. The LD increased serum cholesterol by 47% compared to the standard diet. BHA in combination with the LD markedly increased serum cholesterol levels 2 fold higher compared to LD alone. OA in combination with the LD increased serum cholesterol levels by 15%. Together the data demonstrate that Nrf2 activators increased liver weight along with serum cholesterol levels when combined with a high cholesterol diet. (NIH 5K22ES013782, 3R01ES016042-02S1).

PS 1117 BERBERINE INDUCES HEPATIC STEATOSIS *VIA* C/EBPβ ACTIVATION.

B. Lee[1], Y. Choi[1], H. Suh[1], S. Jeoung[1] and K. Lee[2]. *[1]Seoul National University, Seoul, Republic of Korea and [2]Sungkyunkwan University, Suwon, Republic of Korea.*

Berberine (BBR) is a isoquinoline alkaloid with wide potential therapeutic properties against diabetic and obesity. BBR improves lipid metabolism in obese and diabetic rodent models via activation of AMP-activated protein kinase. However, the hepatotoxicity of BBR in terms of the hepatic lipid metabolism is not clearly understood. Here we show that BBR increased fatty acid uptake and caused lipid accumulation in HepG2 cells. BBR treatment induced lipid accumulation in HepG2 cells as determined by nile red staining. BBR stimulated CCAAT/enhancer-binding protein β (C/EBPβ) expression in a dose-dependent manner and its downstream molecule peroxisome proliferator-activated receptor γ and CD36 was also increased. Upregulation of CD36, a fatty acid transporter, induced by BBR facilitated the uptake of fatty acid determined by BODIPY-C16. Conversely, C/EBPβ RNA interference attenuated BBR-induced lipid accumulation and uptake of fatty acid. These data provide evidence that BBR can cause fatty liver disease mediated by C/EBPβ.

PS 1118 EFFECTS OF MEDICINAL HERBS AND THEIR COMPONENTS ON THE STEATOGENIC HEPATOTOXICITY.

Y. Choi[1], S. Jeoung[1], H. Suh[1], H. Choi[1], S. Park[1], S. Oh[1], J. Choi[2], S. Lee[3], K. Lee[4] and B. Lee[1]. *[1]Seoul National University, Seoul, Republic of Korea, [2]Pukyong National University, Pusan, Republic of Korea, [3]Yeungnam University, Gyeongbuk, Republic of Korea and [4]Sungkyunkwan University, Suwon, Republic of Korea.*

Herbal medicine is widely used in many countries for the treatment of many diseases. Although the use of herbal extract as alternative medicine is even growing, their toxicological properties have not been thoroughly investigated. In this study, we investigated the effect of water and ethanol extracts of 10 herbs on the hepatic lipid metabolism causing steatogenic hepatotoxicity. Ethanol extracts of Cirsium japonicum, Casthamus tincorius and Corydalis ternata Nakai induced the lipid accumulation in Sk-hep1 human hepatoma cells as determined by nile red staining. They increased the luciferase activity of SRE and decreased that of PPRE, indicating the possibility of the enhanced fatty acid synthesis along with the decreased fatty acid oxidation. To identify the components responsible for the fat accumulation, we tested 12 chemicals isolated from the three herbs, apigenin, luteolin, pectolinarin and lupeol from Cirsium japonicum, carthamin, terpinene, camphor and terpineol from Casthamus tincorius and canadine, tetrahydrocoptisine, thalifendine and berberine from Corydalis ternata Nakai increased the accumulation of lipid droplet significantly. These results suggest that ethanol extracts of Cirsium japonicum, Casthamus tincorius and Corydalis ternata Nakai can cause fatty liver disease and it is mediated by decrease of β-oxidation of fatty acid and increase of lipogenesis.

PS 1119 FIBRINOGEN DEFICIENCY INCREASES LIVER INJURY AND EARLY GROWTH RESPONSE-1 EXPRESSION IN A MODEL OF CHRONIC XENOBIOTIC-INDUCED CHOLESTASIS.

J. P. Luyendyk[1], K. M. Kassel[1], K. Allen[1], G. L. Guo[1], G. Li[1], G. H. Cantor[2] and B. L. Copple[1]. *[1]Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, Kansas City, KS and [2]Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ.*

Chronic cholestatic liver injury is associated with hepatic fibrin deposition. Key components of the fibrinolytic pathway modulate cholestatic liver injury by regulating activation of hepatocyte growth factor (HGF). However, the exact role of hepatic fibrin deposition in chronic cholestasis is not known. We tested the hypothesis that hepatic fibrin limits liver injury induced by cholestasis. Fibrinogen (Fbg)-deficient mice (Fbgα-/- mice) and heterozygous control mice (Fbgα+/- mice) were fed either control diet, or a diet containing 0.025% alpha-naphthylisothiocyanate (ANIT), which selectively injures bile duct epithelial cells in the liver, for two weeks. Hepatic fibrin and collagen deposits were evident around intrahepatic bile ducts in livers of heterozygous control mice fed the ANIT diet. Complete Fbg-deficiency was associated with elevated serum bile acids, periportal necrosis, and increased serum ALT activity in mice fed the ANIT diet. Fbg-deficiency was associated with enhanced hepatic expression of the transcription factor Egr-1 and enhanced induction of genes encoding the Egr-1-regulated pro-inflammatory chemokines MCP-1, KC/Gro and MIP-2. Interestingly, peribiliary collagen deposition was not evident near necrotic areas in Fbg-deficient mice. Taken together, the results suggest that in this model of chronic cholestasis, fibrin constrains the release of bile constituents from injured intrahepatic bile ducts, thereby limiting the progression of hepatic inflammation and hepatocellular injury.

PS 1120 ENDOGENOUS CYP1B1 METABOLISM *IN VIVO* CONTROLS ENDOGENOUS LIVER PPAR ACTIVITY WITHOUT EXPRESSION IN HEPATOCYTES.

J. R. Bushkofsky[1, 2], M. L. Larsen[3], S. Wang[3] and C. R. Jefcoate[1, 2, 3]. *[1]Endocrinology and Reproductive Physiology, University of Wisconsin-Madison School of Medicine and Public Health, Madison, WI, [2]Molecular and Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI and [3]Department of Pharmacology, University of Wisconsin-Madison, Madison, WI.*

Cytochrome P4501b1 (Cyp1b1) is known as a key mediator in the activation of PAHs to reactive metabolites that initiate cancer and effect immunosuppression. Cyp1b1 is highly expressed in multipotential progenitor cells, in endothelia from various tissues and in macrophage. In endothelia, Cyp1b1 deletion produces increased oxidative stress, which then disrupts capillary morphogenesis, a model of angiogenesis. This disruption is reversed by lowering oxygen levels and by addition of N-acetyl cysteine. Cyp1b1 deletion in mice suppressed diet-induced adiposity, improved insulin sensitivity and blocked hepatic steatosis caused by high dietary fat. Although scarcely expressed in hepatocytes, expression of over 2,000 genes was affected in the liver ($p<0.025$). An appreciable number of responses to high dietary fat are mimicked by Cyp1b1 deletion, while others such as SCD1 suppression are appreciably enhanced by the combination. Among these responses, 31/33 Cyps that are detectable in liver responded to either dietary fat, Cyp1b1 deletion or the combination. This group includes two of the most responsive genes, Cyp4a10 and Cyp4a14, which catalyze fatty acid ω-hydroxylation. One of the largest responses is a 6-fold suppression of PPARγ by Cyp1b1 deletion, accompanied by near complete loss of constitutive expression of 30 genes that respond to activation of PPARα. Liver PPARγ stimulates hepatic lipogenesis, while PPARα stimulates genes associated with fatty acid metabolism. Constitutive PPARγ expression and PPARa activation may therefore depend on Cyp1b1 metabolism outside the hepatocyte. The loss of PPAR activity may account for the surprisingly low oxidative stress and steatosis. A model is developed in which Cyp1b1-deficiency in non-parenchymal cells of liver sinusoids modifies signaling to hepatocytes.

PS 1121 EVIDENCE OF AhR-DEPENDENT SUPPRESSION OF IMMUNOGLOBULIN EXPRESSION.

M. J. Wourms and C. Sulentic. *Pharmacology and Toxicology, Boonshoft School of Medicine Wright State University, Dayton, OH.*

Inhibition of the antibody forming cell response is one of the most sensitive endpoints of dioxin immunotoxicity. 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD) inhibits immunoglobulin (Ig) expression in mouse B-cells which correlates with a decrease in Ig heavy (IgH) chain transcripts. Transcription of the IgH chain gene involves several regulatory elements including the 3'IgH regulatory region (3'IgHRR). We have previously identified dioxin responsive elements (DRE) in the 3'IgHRR capable of binding the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor known to regulate dioxin sensitive genes. We hypothesize that TCDD-activated AhR inhibits Ig expression by interacting with the 3'IgHRR. In our current study we utilized an IgA secreting mouse B-cell line that stably expresses a 3'IgHRR-regulated transgene. We obtained an approximately 50% reduction of AhR expression by lentiviral-mediated gene delivery of AhR shRNA. To inhibit residual AhR activity, we employed a competitive AhR antagonist (CH-223191) which reduced Cyp1A1 (the hallmark of a functional ligand-activated AhR) expression by up to 80% in the AhR knockdown cells. TCDD-induced suppression of the 3'IgHRR-regulated transgene and of endogenous IgA protein expression was significantly reduced during co-treatment with LPS and TCDD in the AhR-knockdown cell population following pretreatment with CH-223191.

These results confirmed our hypothesis that by inhibitory interactions at the 3'IgHRR, AhR suppresses Ig expression during TCDD treatment. Interestingly, basal expression of both the 3'IgHRR-regulated transgene and IgA was increased in the AhR-knockdown cell population suggesting a role of AhR independent of ligand-activation. Ongoing studies are focused on determining the DRE target of TCDD-activated AhR in the 3'IgHRR and elucidating the involvement of AhR in basal Ig expression which could reveal a novel physiological role of AhR.
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PS 1122 TCDD-INDUCED MODULATION OF THE hs1, 2 ENHANCER WITHIN THE 3'IgHRR IN MOUSE AND HUMAN.

J. Liu, T. Fernando, S. Ochs and C. E. Sulentic. *Pharmacology and Toxicology, Boonshoft School of Medicine, Wright State Unversity, Dayton, OH.*

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental toxin known to suppress immunoglobulin (Ig) gene expression and antibody secretion. It has been suggested that the inhibition of B cell function by TCDD is transcriptionally mediated through the aryl hydrocarbon receptor (AhR) and binding to dioxin response elements (DRE). Transcriptional regulation of the Ig heavy chain (IgH) involves the 3'IgH regulatory region (3'IgHRR) and its enhancers (hs3, hs1,2, and hs4), which contain DNA binding sites for several transcription factors including NF-κB and the AhR. Previously our lab has demonstrated TCDD-induced binding of the AhR complex to a DRE in both hs1,2 and hs4 enhancers as well as a marked inhibition of the mouse 3'IgHRR in a CH12.LX B-cell line. Interestingly, in humans, a polymorphism of the hs1,2 enhancer results in a varying number of tandem repeats of a 53 bp sequence which has been correlated with autoimmune diseases such as IgA nephropathy and Celiac disease. The human hs1,2 enhancer has binding sites for κB and DRE; however, Pax5 and NF-αP, which are important regulators for the mouse hs1,2 enhancer, are not present. The purpose of our study is to comparatively evaluate the effect of TCDD and LPS on human and mouse hs1,2 enhancers in a mouse (CH12.LX) and a human (IM-9) B cell line. Using transient luciferase assays, TCDD markedly enhanced human hs1,2 activity and even augmented LPS-induced activation in LPS-stimulated CH12.LX mouse B cells. This starkly contrasted with the TCDD-induced inhibition of the mouse hs1,2 enhancer and 3'IgHRR. Moreover, we observed a similar activation of the human hs1,2 enhancer by TCDD in the IM9 human B-cell line. Mutational analysis is underway to evaluate the impact of different binding sites within the human and mouse hs1,2 enhancers on TCDD-induced transcriptional modulation. Taken together, our data suggest that the hs1,2 enhancer is a significant target of TCDD and support species differences in transcriptional regulation of the hs1,2.(Supported by NIEHS R01ES014676,R01ES014676-03S2)

PS 1123 PAX5 MAY MEDIATE TCDD-INDUCED DIFFERENCES IN TRANSCRIPTIONAL REGULATION OF THE MOUSE AND HUMAN hs1, 2 ENHANCER.

S. Ochs, J. Liu and C. Sulentic. *Pharmacology and Toxicology, Wright State Unversity, Dayton, OH.*

Immunoglobulin heavy chain (IgH) expression and Ig secretion is inhibited by the environmental contaminant 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD). Within the IgH gene, the 3' IgH regulatory region (3'IgHRR) has been identified as a transcriptional target of TCDD. The 3'IgHRR, which in part regulates transcription of the IgH gene, is composed of four enhancers in the mouse: hs3a; hs1,2; h3b; hs4 and three enhancers in the human: hs3a; hs1,2; hs4. Interestingly, a repeating polymorphism, approximately 53 bp in length, within the human hs1,2 enhancer, has been correlated with several autoimmune disorders. TCDD induces a decrease in transcriptional activity of the mouse hs1,2 enhancer, while the human polymorphic hs1,2 enhancer is increased. The purpose of the current study is to determine if this opposing result may be partially due to Pax5, a regulator of B-cell maturation that is down-regulated at the plasma cell stage to allow IgH transcription and Ig secretion. Two Pax5 binding sites are present in the mouse hs1,2 whereas none are present in the human hs1,2 and TCDD has been shown to interfere with the down-regulation of Pax5. Utilizing site-directed mutagenesis, a Pax5 binding site identical to the mouse was inserted into a human hs1,2 enhancer reporter plasmid. Transient transfection studies using a mouse (CH12.LX) and human (IM-9) B cell line expressing functional AhR signaling demonstrated a decrease in overall TCDD-induced transcriptional activity with the insertion of a Pax5 site within the human hs1,2 enhancer. These results suggest Pax5 may be in part responsible for the differing transcriptional regulation between the mouse and human hs1,2 enhancer following TCDD treatment. Additionally, species specific sequence differences may complicate the translation of mouse studies to humans. (Supported by NIEHS R01ES014676)

PS 1124 ROLE OF THE 3'IgHRR IN TCDD-INDUCED SUPPRESSION OF THE IMMUNOGLOBULIN HEAVY CHAIN.

J. L. Panchal, E. Romer, T. Fernando and C. Sulentic. *Pharmacology and Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, OH.*

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) inhibits antibody secretion and immunoglobulin heavy chain (IgH) expression. Our previous work has shown that a possible mechanism for inhibition of IgH expression could be inhibition of the 3'IgH regulatory region (3'IgHRR). The 3'IgHRR has four enhancer regions (hs3a; hs1,2; hs3b; hs4) which are purported to control IgH gene expression. Previously we demonstrated a sensitive inhibition by TCDD of LPS-induced 3'IgHRR activity which is correlated with an inhibition of LPS-induced hs1,2 enhancer activity; however, the hs4 enhancer showed TCDD-induced activation following the co-treatment of TCDD and LPS. Therefore, the objective of the current study was to determine if the hs1,2 enhancer mediates the inhibitory effect of TCDD on 3'IgHRR activation. We generated a CH12.LX cell line stably expressing a γ2b transgene under the regulation of the 3'IgHRR and containing loxp sites flanking either the hs3a/hs1,2 or the hs3b/hs4 enhancer pairs. Transient transfection with Cre recombinase results in recombination at the loxp sites and deletion of the appropriate enhancer pair. Cells expressing Cre recombinase were sorted and 96 clones from each parental (i.e., loxp flanking hs3a/hs1,2 or hs3b/hs4) were analyzed by PCR for successful recombination. We found 8 clones and 6 clones with successful deletion of the hs3a/hs1,2 and hs3b/hs4 respectively. Preliminary analysis showed inhibition of the hs3a/hs1,2 enhancer pair and no effect to inhibition of the hs3b/hs4 enhancer pair. These results suggest that the hs1,2 is the primary mediator of TCDD-induced 3'IgHRR inhibition and that the hs4 enhancer in combination with the hs3b enhancer is affected differently than when the hs4 is analyzed in isolation or by transient transfection. A polymorphism of the hs1,2 enhancer is correlated with autoimmune diseases like IgA nephropathy and Celiac disease. Hence modulation of hs1,2 enhancer activity by TCDD may influence the initiation or progression of these diseases. (Supported by NIEHS R01ES014676)

PS 1125 NF-κB/REL AND THE AhR IN MODULATING THE 3′IgHRR.

R. Salisbury and C. Sulentic. *Pharmacology and Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, OH.*

Transcriptional regulation of the immunoglobulin heavy chain (IgH) gene involves several regulatory elements including the 3′IgH regulatory region (3′IgHRR). The mouse 3′IgHRR is composed of at least four enhancers (hs3A; hs1,2; hs3B; hs4) and contains binding sites for several transcription factors including NF-κB/Rel proteins and dioxin responsive elements (DRE). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a disrupter of B-cell differentiation and Ig production, induces binding of the aryl hydrocarbon receptor (AhR) to a DRE motif within both the hs1,2 and hs4 enhancers. Interestingly, TCDD profoundly inhibits 3′IgHRR activation induced by the B-cell activator lipopolysaccharide (LPS), but enhances the activation of the hs4 enhancer in transient luciferase reporter studies. Within the hs4 enhancer, the DRE overlaps an NF-κB/Rel binding site suggesting that both the AhR and the NF-κB together play a role in overall modulation of the 3′IgHRR. The objective of the current study was to evaluate the role of NF-κB/Rel and the AhR following LPS or CpG (activators of the NF-κB pathway) stimulation and TCDD treatment on 3′IgHRR and hs4 activity. In our studies we utilized the CH12.LX B cell line, the CH12.IκBαAA cell line which expresses an inducible IκBα super repressor (IκBαAA), and the CH12.γ2b-3′IgH cell line that stably expresses a γ2b-3′IgHRR reporter. Utilizing transiently expressed luciferase reporters, we found that induction of IκBαAA expression partially attenuated both LPS and CpG-induced activation of the 3'IgHRR and hs4 and partially reversed the effects of a TCDD and LPS/CpG co-treatment on the activity of the 3'IgHRR and hs4. Furthermore, the addition of an AhR antagonist, CH223191, markedly reversed TCDD-induced inhibition of the LPS-activated 3'IgHRR and inhibited the synergistic activation of the hs4. Co-immunoprecipitation assays demonstrated an interaction between the AhR and NF-κB/Rel. These results suggest that the NF-κB/Rel proteins, perhaps through an interaction with the AhR, are partially responsible for 3′IgHRR modulation by LPS/CpG and TCDD. Supported by NIEHS R01ES014676)

PS 1126 PERSISTENT DEVELOPMENTAL ARYLHYDROCARBON RECEPTOR ACTIVATION MODULATES NOTCH1-DEPENDENT T-CELL BUT NOT B-CELL DIFFERENTIATION POTENTIAL.

L. Ahrenhoerster, P. Lakatos and M. Laiosa. *School of Public Health, University of Wisconsin Milwaukee, Milwaukee, WI.*

Arylhydrocarbon receptor (AHR) activation in the immune system has generated renewed interest with the findings that the AHR is an important regulator of hematopoiesis and regulatory T-cell differentiation. In order to understand the mechanism(s) underlying precursor cell fate decisions potentially impacted by AHR signal transduction, our laboratory has tested the capacity of undifferentiated mouse fetal liver progenitors to respond to signals known to drive development into the B or T cell lineage. Specifically, lineage negative, cKit+, Sca-1+ (LSK) multipotent hematopoietic progenitor cells obtained from gestational day (GD) 14.5 mouse fetal livers were placed in culture with either OP9-GFP cells which drives B-cell development or OP9-DL1 stromal cells, which provide a Notch signal driving differentiation to the T-cell lineage. To test the hypothesis that persistent AHR activation during development modulates LSK differentiation capacity, mice were exposed to 5μg/kg 2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD) in olive oil on GD 0 and 7 by gavage or vehicle control. TCDD is a ubiquitous environmental contaminant and potent AHR agonist, which humans are exposed to daily through low level food contamination. On day 14.5, fetal livers were dissected, LSKs purified by cell sorting, and subsequently placed into culture. At the end of the 12-day culture period, cells were enumerated and the proportion of T-cells or B-cells obtained from each co-culture was determined by flow cytometry. While an equivalent number of B-cells were obtained from control or TCDD exposed mice, the number of T-cells generated in culture was reduced by nearly 40% in the cultures derived from TCDD exposed pregnant dams. These data suggest that early life AHR activation reprograms a multipotent progenitor's capacity to mediate Notch1-dependent T-cell differentiation.

PS 1127 THE ROLE OF THE ARYL HYDROCARBON RECEPTOR IN WOOD SMOKE-INDUCED MACROPHAGE SUPPRESSION.

C. T. Migliaccio, E. Kobos, V. Porter and T. Ward. *Center for Environmental Health Sciences, University of Montana, Missoula, MT.*

Alveolar macrophages (AM) are part of the first line of defense against pulmonary infections. AM are responsible for clearing particles, such as silica or bacteria, and are key regulatory cells of the immune response in the airways. In addition to decreases in overall AM function, including antigen presentation and bacterial clearance, we observed a lack of classic inflammation (i.e. TNFα, neutrophil influx, increases in total protein) following acute exposure to WS. One potential explanation for these decreases is an observed link between polycyclic aromatic hydrocarbons (PAH), RelB activation, and the suppression of macrophage-guided inflammation. RelB, the non-canonical NFκB pathway, has been associated with decreases in inflammation and apoptosis, both of which have been observed following exposure to WS. In order to determine whether the non-canonical pathway is being activated, AM were analyzed for RelB translocation to the nucleus using immunohistochemistry. We observed that both WS and concentrated WS-derived particulate matter (WS-PM) induced activation of RelB, where activation appears dependant on components associated with the water-insoluble fraction (IF) of concentrated WS-PM. Activation of RelB can occur in response to PAHs, via the aryl hydrocarbon receptor (AhR), and these mechanisms have been linked to macrophage regulation. To this end the PAH analysis of WS-PM detected 4-5 PAHs only in the IF of WS-PM. Further analysis showed specific AhR activation by the IF, at levels significantly higher than controls. These data suggest a role for specific AhR activation in the WS-induced suppression of AM.
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PS 1128 TCDD TREATMENT ENHANCES THE M1 PHENOTYPE IN J774 MURINE MACROPHAGES.

A. E. McCartney, R. A. Aguayo, P. V. Hamilton and K. A. Mitchell. *Biological Sciences, Boise State University, Boise, ID.*

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent environmental contaminant that elicits toxicity by activating the aryl hydrocarbon receptor. Although the mechanism of toxicity is unclear, exposure to TCDD has been shown to enhance inflammation in several model systems. A typical inflammatory response depends on the production of soluble mediators by activated macrophages, which fall into two phenotypically distinct categories: classically activated M1 (inflammatory) macrophages and alternatively activated M2 (rebuilding) macrophages. M1 macrophages produce iNOS and soluble mediators such as TNF-α and IL-6, which may exacerbate inflammation, whereas M2 macrophages produce mediators that curtail inflammation and promote wound healing. In this study, we tested the hypothesis that TCDD treatment skews macrophage activation towards the M1 phenotype. Murine J774 macrophages were primed for 24 hr with either IFNγ, which drives an M1 phenotype, or IL-4/IL-13, which drives an M2 phenotype, in the presence or absence of TCDD (10 nM). Cells were then stimulated with LPS (50 ng/mL) for 24 hours. Cell lysates and supernatants were analyzed to measure TNF-α, IL-6, iNOS, and CCR7, which are characteristic of M1 macrophages. In J774 cells primed with IFNγ, TCDD had no effect on CCR7 expression or cytokine production, but it increased iNOS expression. Treatment of J774 cells with IL-4 and IL-13 suppressed M1 activation markers and cytokines in vehicle-treated cells but not in TCDD-treated cells. When these cells were subsequently stimulated with LPS, TCDD-treated cells expressed levels of IL-6 that were ten-fold higher than vehicle controls. These results indicate that TCDD treatment may enhance the activation of M1 macrophages and skew macrophages activated under M2 conditions toward the M1 phenotype. Moreover, the specific cell signaling pathways in macrophages that are enhanced by TCDD appear to be confined to those that drive iNOS expression and IL-6 production.

PS 1129 THE ARYL HYDROCARBON RECEPTOR MODULATES PHENOTYPE AND FUNCTION OF DENDRITIC CELLS.

G. Jin and B. Lawrence. *Environmental Medicine, University of Rochester, Rochester, NY.*

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that is expressed in cells of the immune system. AhR activation by a variety of ligands is profoundly immunomodulatory, with changes in CD8+ T cell function and impaired ability to fight influenza virus infection being particularly sensitive to perturbation by the pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a well-known high affinity AhR agonist. We have previously reported that AhR activation by TCDD reduces the proliferation and differentiation of influenza virus-specific CD8+ T cells in the mediastinal lymph nodes (MLN). However, AhR activation does not directly affect proliferation and differentiation of CD8+ T cells, suggesting that defects in accessory cells such as antigen presenting cells (APCs) are directly affected by AhR activation. Dendritic cells (DCs) are professional APCs. In response to infection, they capture antigen in the lung, migrate to the MLN and play a key role in the priming of virus-specific naïve CD8+ T cells. In this study, we investigated the effects of AhR activation by TCDD on the function and phenotypic profile of DCs in influenza virus-infected mice. We show that AhR activation changes the number of functionally distinct DC subsets in the lung and MLN, and reduces the ability of DCs to stimulate naïve CD8+ T cells in vitro. In particular, AhR activation reduces cell number of CD103+ DCs in the MLN. This is important because our data and work of others indicate that CD103+ DCs are superior in priming virus specific naïve CD8+ T cells. Our results indicate that AhR activation by TCDD modulates DC phenotype and function, and suggest that environmental factors may contribute to differential susceptibilities and responses to respiratory viral infections.
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PS 1130 MODULATION OF NF-KB MEDIATED DENDRITIC CELL DIFFERENTIATION AND FUNCTION BY ARYL HYDROCARBON RECEPTOR ACTIVATION.

C. Vogel[1, 3], S. Goth[2], D. Wu[3], V. Kou[3], A. Lollies[3], I. Pessah[2] and F. Matsumura[1, 3]. [1]*Environmental Toxicology, University of California, Davis, CA,* [2]*Molecular Biosciences, University of California, Davis, CA and* [3]*Center for Health and the Environment, University of California, Davis, CA.*

Recent findings indicate that the aryl hydrocarbon receptor (AhR) plays an important role in the immune system including T cell and dendritic cell (DC) differentiation. Here we show that activation of AhR by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) lead to a time-dependent induction of LPS-activated target genes in human U937 DC like IL-8 and CCL1, in contrast expression of CCL4 and CCL18 are antagonized by AhR activation. The DC surface marker CD86 was increased by both LPS and TCDD and is synergistically increased after LPS/TCDD cotreatment. The data also indicate that this synergy involves AhR and RelB. On the other hand, the expression of CD40 and CD80 was only significantly increased by LPS in U937 DC, but did not change by TCDD. However, the LPS-induced expression of CD80 and CD40 was drastically suppressed in presence of TCDD. Furthermore, TCDD induced expression of both isoforms of the regulatory enzyme indoleamine 2,3-dioxygenase (IDO1 and IDO2) which has immunomodulatory activity by increasing the number of regulatory T cells. Results show that in contrast to TCDD, treatment with Interferon-(IFN)γ or 6-formylindolo[3,2-b]carbazole (FICZ) may induce IDO1 but not the expression or promoter activity of

IDO2. Similar effect by TCDD and LPS were found in inflammatory bone marrow derived DC (BMDC). These data indicate that the cross-talk between AhR and NF-κB is not limited to chemokines and cytokines but also affects significantly the expression of surface markers critical for the differentiation and function of DC. Data also show that AhR activation may drastically change the regular NF-κB-mediated inflammatory response in a gene target dependent manner. For instance the usually transient effect of LPS can be significantly increased and prolonged through the activation of AhR.

PS 1131 AH RECEPTOR ACTIVATION GENERATES REGULATORY DENDRITIC CELLS CAPABLE OF INDUCING CD4+ CD25+ FOXP3+ REGULATORY T CELLS.

T. Simones, J. Bankoti and D. M. Shepherd. *Center for Environmental Health Sciences, University of Montana, Missoula, MT.*

The Aryl hydrocarbon Receptor (AhR) mediates the toxic effects of various environmental and dietary compounds and has recently been shown to contribute to the generation of regulatory T cells (Tregs). However, the mechanisms underlying Treg induction and immunomodulation following AhR activation remain to be defined. Within the immune system, dendritic cells (DCs) express high levels of the AhR and are sensitive to AhR activation. We hypothesized that AhR activation in DCs leads to the generation of regulatory DCs and subsequent Treg induction. To test this hypothesis, murine GM-CSF bone marrow-derived DCs (BMDCs) were generated in the presence of TCDD or a vehicle control. AhR activation significantly increased the expression of the regulatory genes, TGFβ3 and indoleamine 2,3-dioxygenase-1 and -2 (IDO1, IDO2) but not IL-27, OX40L or Aldh1a2 in unstimulated BMDCs. Additionally, TGFβ1, TGFβ2, IDO1 and IDO2 expression increased, while IL-27, OX40L and Aldh1a2 levels remained unchanged in LPS-stimulated AhR-activated BMDCs. Under antigen-specific conditions, AhR-activated BMDCs generated an increased frequency of CD4+ CD25+ FoxP3+ Tregs after three days in culture with media alone or IL-2/TGFβ supplemented media, thereby demonstrating the functional capacity of AhR-induced regulatory DCs. In the absence of antigen, TCDD-treated regulatory BMDCs led to a sustained maintenance of Tregs. Conversely, production of effector T cell cytokines including IL-2, IFN-γ and IL-10 was inhibited in these cultures. Overall, AhR activation induces regulatory DCs capable of directly inducing CD4+ CD25+ FoxP3+ Tregs in vitro. Studies are ongoing to determine the specific mechanisms (i.e. cell contact, TGFβ and/or IDO dependency) of Treg induction by AhR-activated regulatory DCs. This novel research significantly advances our understanding of AhR-induced immunomodulation and provides a foundation for the generation of AhR-targeted, antigen-specific therapeutics.

PS 1132 ALL AHR LIGANDS ARE NOT CREATED EQUAL: THE DURATION OF AHR ACTIVATION IS A KEY PARAMETER IN MODULATING THE IMMUNE RESPONSE TO INFLUENZA VIRUS INFECTION.

J. Head[1] and B. Lawrence[1, 2]. [1]*Environmental Medicine, University of Rochester, Rochester, NY and* [2]*Microbiology and Immunology, University of Rochester, Rochester, NY.*

It is apparent that the aryl hydrocarbon receptor (AhR) plays a role in a variety of immunological pathways via multiple targets. While many studies have traditionally utilized 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to activate the receptor, other AhR ligands have also been shown to affect various immune endpoints. Interestingly, activation of the AhR can have either host-beneficial or detrimental consequences depending on the type of AhR ligand used. One significant difference between many AhR agonists is the duration of time in which they activate the receptor. Our laboratory set out to examine whether the duration of AhR activation is responsible for differentially impacting AhR-sensitive immunological endpoints using two different AhR agonists: TCDD and the tryptophan degradation product 6-formylindolo[3,2-*b*]carbazole (FICZ). Although FICZ has a similar affinity for the AhR as TCDD, it has a much shorter half-life *in vivo*. We used FICZ to examine the contribution of duration of AhR activation on the immune response to influenza virus infection, a system with well-characterized, AhR-induced changes in mice treated with TCDD. We show here that transient AhR activation caused by a single dose of FICZ did not have the same impact on the immune response to influenza virus as a single dose of TCDD. Interestingly, even when AhR activation was prolonged during infection using constant delivery of FICZ with mini-osmotic pumps, the immunomodulatory changes observed with a single dose of TCDD were not replicated. These findings suggest that although duration of activation may contribute to the AhR's effects on immune function, it is likely that additional ligand-specific parameters also play an integral part in dictating how the AhR modulates immune function. Determining these specific parameters is key to fully understanding the physiological role the AhR plays as well as detailing the varied molecular mechanisms by which the AhR perturbs immune pathways.

PS 1133 TCDD AMELIORATES COLITIS IN MICE BY INDUCTION OF REGULATORY T CELLS (TREGS) *VIA* AHR ACTIVATION.

N. P. Singh[1], U. Singh[1], B. Singh[2], L. Hofseth[3], M. Nagarkatti[1] and P. Nagarkatti[1]. [1]*Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC,* [2]*Primate Research Center, Emory University, Atlanta, GA and* [3]*South Carolina College of Pharmacy, University of South Carolina, Columbia, SC.*

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a potent environmental pollutant that activates aryl hydrocarbon receptor (AhR), which in turn regulates T cell differentiation. Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC), are associated with chronic inflammation of the intestinal tract. In the current study, we investigated the effect of AhR activation on Dextran Sodium Sulphate (DSS)-induced colitis in mice. Colitis was generated in mice (C57BL/6) administering 3% DSS in water by ad libitum. The mice were treated with vehicle (corn oil) or TCDD in corn oil (25 micro gram/kg body weight) and development of clinical symptoms was followed for two weeks. AhR activation suppressed the clinical score of colitis as well as various pathological markers, including the loss of body weight. AhR activation during colitis also caused decreased levels of inflammatory cytokines (IL-6 and TNF-alpha) and chemokines (MCP-1, KC, and Eotaxin) in the sera. Also, percentage of CXCR3+ T cells in lamina propria (LP) decreased while the percentage of myeloid derived suppressor cells (MDSCs) increased both in spleen and LP. We also noted induction of both systemic and mucosal FoxP3+ regulatory T cells (Tregs). AhR activation in vitro also led to increased differentiation of Tregs. Furthermore, antigen-specific activated T cells also showed significant differentiation into Tregs in the presence of TCDD. Together, these results suggested that TCDD via AhR activation in vivo decreases CXCR3+ T cells, induces FoxP3+Tregs, and increases mucosal MDSC expression which in turn may inhibit inflammatory Th1 cells and suppress the intestinal inflammation. Together, the current study demonstrates that AhR activation may serve as a novel therapeutic target for the treatment of chronic inflammatory bowel disease (Supported in part by NIH grants R01ES09098, P01AT003961, R01ES019313).

PS 1134 ARYL HYDROCARBON RECEPTOR ACTIVATION SUPPRESSES INTESTINAL INFLAMMATION.

J. M. Benson and D. M. Shepherd. *Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT.*

Exaggerated T cell-mediated immune responses to commensal bacteria arise from both genetic and environmental factors and ultimately lead to Crohn's disease, a chronic inflammatory disease of the gastrointestinal tract. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the potent ligand of the aryl hydrocarbon receptor (AhR), has been shown to generate regulatory T cells (Tregs) that suppress immune responses. We hypothesized that AhR activation reduces disease severity and inflammation in the gut. The 2,4,6-trinitrobenzenesulfonic acid (TNBS) model of colitis was used to test this hypothesis. Mice were gavaged with TCDD prior to colitis induction with TNBS, which included peripheral sensitization before enema administration to elicit efficient effector T cell responses. The extent of colonic inflammation was determined by assessing severity of clinical symptoms, tissue damage, and inflammatory cell infiltration in the colon and surrounding lymph tissue. TCDD-treated mice recovered more rapidly, experienced less colonic inflammation, and decreased pro-inflammatory mediator production compared to vehicle-treated mice. Increased frequencies of Foxp3+ Tregs were also observed in the gut of TNBS-exposed Foxp3egfp mice. In these mice, the CD103+ regulatory dendritic cell (DC) population was also increased. Regulatory genes, such as idoleamine-2,3,-dioxygenase, were elevated while levels of pro-inflammatory genes, such as IL-17, were reduced in the colon. Furthermore, in vitro studies conducted on intestinal epithelial cells and bone marrow-derived DCs demonstrated that TCDD suppresses LPS-induced immune responses suggesting that these cells may be critical to suppressing gut inflammation in vivo. Collectively, these results indicate that AhR activation by TCDD generates a regulatory environment in the gut that decreases inflammation in a murine model of colitis. This research was supported by the grants P20RR017670 (NCRR), P20RR015583(NCRR), ESO13784(NIH), and F31AT005557(NCCAM).

PS 1135 BACH2 BINDING TO P*rdm1* AS A MECHANISM OF 2, 3, 7, 8-TETRACHLORODIBENZO-*P*-DIOXIN(TCDD)-MEDIATED IMMUNE SUPPRESSION.

A. S. Phadnis[1, 3], K. DeAbrew[4], R. S. Thomas[4] and N. E. Kaminski[2, 3]. [1]*Genetics Program, Michigan State University, East Lansing, MI,* [2]*Pharmacology & Toxicology, Michigan State University, East Lansing, MI,* [3]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [4]*Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Altered B cell differentiation and decreased IgM production are some of the major AHR-dependent effects of TCDD seen both in vitro and in vivo. Previously, using a genome-wide analysis of ligand-dependent AHR DNA binding, we identified Bach2 as a novel gene directly regulated by the AHR in a murine B lymphoma cell line CH12.LX. Bach2 is a B cell specific repressor of P*rdm1* and mediates its repressive functions by binding to Maf recognition elements (MAREs) in the regulatory regions of P*rdm1*. qRT-PCR experiments performed in resting and LPS-activated CH12.LX cells showed induction of Bach2 mRNA levels at 2h in presence of TCDD; concordant with the chromatin immunoprecipitation and gene expression microarray experiments. To further elucidate the role of Bach2 in regulating P*rdm1*, electrophoretic mobility shift assays were performed on nuclear extracts from LPS-activated and resting CH12.LX cells treated with TCDD. In both P*rdm1* promoter and intron 5 MAREs, increased TCDD-inducible DNA binding activity of Bach2 was seen at 4h in LPS-activated cells as compared to treatment with LPS or TCDD alone. A TCDD-concentration dependent DNA binding activity was also observed at the intron5 MARE. In contrast, maximum DNA-binding was seen at 0.1nM TCDD in the promoter MARE at 4h post-treatment. In order to identify the MARE- binding protein complex, supershift assays were performed using an anti-Bach2 antibody and non-specific goat IgG. Specific Bach2 binding was observed at the intron5 MARE but not at the promoter MARE. These results suggest differential binding activity of Bach2 to the two MARE elements in presence of TCDD. Taken together these findings shed light on a new mechanism of controlling P*rdm1* in TCDD-mediated immune suppression. (Supported by NIH ES04911 and ES02520)

PS 1136 DIDOX (DIHYDROXYBENZOHYDROXAMIC ACID) AMELIORATES MACROPHAGE AHR ACTIVATION, CYP1A1/AHR INDUCTION, AND OXIDATIVE STRESS PROFILES INDUCED BY PCB-126.

C. D. Rice, V. S. Gallichio and T. M. Matsebatlela. *Biological Sciences, Clemson University, Clemson, SC.*

Exposure to AhR-activating xenobiotics is associated with oxidative stress and inflammation. For example, PCB-126 is a potent and well-studied AhR-ligand and inducer of CYP1A1/2 and Phase II metabolic enzymes. Intracellular ROI increases in cells within a few hrs of exposure, long before mRNA transcription and protein induction. Thereafter, levels of ROI continually rise for several days due to a high affinity of PCB-126 for the AhR and recalcitrant metabolism. After a 24 hr exposure of RAW264.7 murine macrophages to 1 μM PCB-126 we show (using qRT PCR) that expression levels of GSH-peroxidase and SOD genes are suppressed, while expression levels of of NADPH-oxidase genes are elevated. As expected, CYP1A1 gene/protein expression is markedly induced and associated with elevated superoxide anion and H2O2 production. In search of strong, but non-toxic antioxidants that may ameliorate AhR-associated ROI and inflammation, we found that Didox (3,4-Dihydroxybenzohydroxamic acid) is very potent. Several GSH-peroxidase genes and GSH reductase, peroxiredoxins, thioredoxin reductase, all three types of SODs, and catalase are elevated by Didox. Furthermore, Didox reverses oxidative stress gene profiles initiated by PCB-126. Of particular note, Didox mimics LPS in that exposure suppresses the expression of both CYP1A1 and AhR genes. We postulate that Didox may have significant potential in ameliorating the toxicity of several classes of AhR-activating immunotoxic compounds.

PS 1137 MECHANISTIC INVESTIGATION OF TCDD EFFECTS ON CD40 LIGAND-INDUCED ACTIVATION OF PRIMARY HUMAN B CELLS: PERTURBATION OF IMMEDIATE AND PERSISTENT SIGNALING.

H. Lu[1, 2], R. Crawford[1, 2] and N. E. Kaminski[1, 2]. [1]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [2]*Pharmacology & Toxicology, Michigan State University, East Lansing, MI.*

The B cell is well established as a sensitive cellular target of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in suppression of the primary antibody response in mice. However, data concerning TCDD effects on human B cells remains scarce. Previous findings that the CD40 ligand-induced IgM response in primary human B cells was decreased by TCDD led us to investigate the mechanisms by which TCDD impairs human B cell effector function using the same in vitro activation model. Critical steps involved in the progression of a resting B cell to a differentiated antibody secreting cells, namely activation, proliferation, and transcriptional regulation of plasmacytic differentiation were assessed using real-time PCR and flow cytometry analyses. TCDD exhibited a modest effect on proliferation, but profoundly attenuated the activation of human B cells, as evidenced by decreased expression of surface activation markers CD80, CD86, and CD69, which was consistently observed among multiple human donors. The impaired activation, which correlated with decreased cell viability, prevented B cells from progressing toward the stage of plasmacytic differentiation. Phosflow techniques were applied to investigate the mechanisms for impairment of B cell activation, and showed that TCDD produced perturbation of immediate and/or persistent activation of mitogen-activated protein kinases, protein kinase B/Akt, signal transducer and activator of transcription 1, 3 and 5, AP-1/c-Jun, and RelA/p65 in activated human B cells. Collectively, this series of study demonstrates that CD40L-induced activation of human B cells is impaired by TCDD, through mechanisms that involve the perturbation of multiple signaling cascades downstream of CD40 and the cytokine receptors. Supported in part by NIH P42 ES04911 and ES02520, the Dow Chemical Company, and a research fellowship from Syngenta AG.

PS 1138 AH RECEPTOR MEDIATED RESTORATION OF SPLEEN AND BONE MARROW FOLLOWING THE CYP1B1-DEPENDENT ADVERSE EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS).

A. U. N'jai[1, 3], M. Larsen[2], C. R. Jefcoate[2, 3] and C. J. Czuprynski[1, 3, 4]. [1]*Pathobiological Sciences, University of Wisconsin, Madison, WI,* [2]*Pharmacology, University of Wisconsin, Madison, WI,* [3]*METC, University of Wisconsin, Madison, WI and* [4]*Food Research Institute, University of Wisconsin, Madison, WI.*

Bone marrow (BM) lymphoid progenitors emigrate to the spleen and thymus for maturation into effector B and T cells, respectively. We have previously reported rapid in vivo suppression of BM progenitors by exposure to certain PAHs. Here, we assess in a coordinated manner the effects of PAHs on lymphoid populations in the bone marrow, blood, spleen and thymus. IP administration of 7,12-dimethylbenz(a)anthracene (DMBA) rapidly suppressed (>50% at 6h) lymphoid CFU-preB progenitor cell activity. By 48 h, cell numbers in the BM, spleen and thymus exhibited similar decreases. Oral administration of DMBA, which results in its clearance within 12h, decreased progenitor cell activity at 6 h but this returned to normal by 48h. IP administration of benzo(a)pyrene (BP) had an adverse effect on BM progenitors that was comparable to DMBA at 6 h. However, this was followed by an Ah Receptor-dependent recovery of progenitor cell activity. By contrast, similar suppression by each PAH was seen for total blood lymphocytes (6h) and thymocytes (48h). The effects of DMBA and BP were both dependent on Cyp1b1 metabolism. BM, spleen, thymus, and blood lymphoid cell populations generally declined and recovered in a temporal pattern similar to the changes noted for BM progenitor cells. These observations are consistent with replenishment of the blood, spleen and thymus populations subsequent to restoration of BM progenitors. Paradoxically, gene expression responses to DMBA were almost absent in BM cells after 12h but were extensive for BP. We infer that Cyp1b1 mediated DMBA metabolism specifically affects the few BM progenitor cells. Many BP gene responses were AhR dependent and may be link to progenitor recovery.

PS 1139 ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR BY TCDD SUPPRESSES SENSITIZATION IN A MOUSE PEANUT ALLERGY MODEL.

V. J. Schulz[1], J. Smit[1, 2], L. Boon[3], M. van den Berg[1], M. van Duursen[1] and R. Pieters[1]. [1]*Immunotoxicology, Institute of Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands,* [2]*Utrecht Center for Food Allergy, Utrecht, Netherlands and* [3]*Bioceros B.V., Utrecht, Netherlands.*

Food allergy is an increasing health problem in Western countries. Previously, it was shown that the intensity of food allergic reactions can be regulated by regulatory T (Treg) cells. In addition, it was shown that activation of the aryl hydrocarbon receptor (AhR) regulates T cells responses and induces Treg cells. Therefore, we investigated whether activation of the AhR could suppress food allergic responses through the induction of Treg cells. C3H/HeOuJ mice received 2,3,7,8-tetrachlorodibenzo-p-dioxin TCDD (0.6, 1.7, 5 and 15 μg/kg BW) orally and were sensitized to peanut by administering peanut extract (PE) by gavage in the presence of cholera toxin (CT). We found that activation of the AhR by TCDD dose-dependently decreased PE-specific IgE, IgG1 and IgG2a. Furthermore, PE-induced IL-5, IL-10 and IL-13 were decreased by TCDD, whereas IFN-γ production was increased. The percentage of Treg cells (CD4+CD25+Foxp3+) increased dose-dependently by TCDD treatment in both spleen and MLN. However, the absolute

number of Treg cells remained the same, whereas the number of CD4+ cells decreased dose-dependently by TCDD. The role of Treg cells in the suppression of the peanut allergic response after AhR activation by TCDD was further investigated by depleting these cells during sensitization to PE with anti-CD25 antibody (i.p.). Depletion of Treg cells reversed the suppressive effect of TCDD on PE-specific antibody levels and PE-induced Th2-cytokine production. Together, these findings showed that activation of the AhR suppressed the peanut allergic response possibly through Treg cells. In addition, our results showed that TCDD treatment shifted the Th1/Th2 cytokine balance towards Th1. These results suggest that the AhR might be a new drug target to treat food allergy. Current studies investigate the mechanism of action of AhR activation on T cell responses during establishment of food allergy.

PS 1140 VALIDATION OF AN *IN VITRO* SCREENING METHOD FOR THE INDUCTION OF DRUG CYTOKINE RELEASE IN CYNOMOLGUS MONKEY.

M. S. Piché, S. Lavallée, S. Legris, R. Falvo, K. Eliacin and L. LeSauteur.
Immunology, Charles River Preclinical Services, Montreal, QC, Canada.

Objectives Changes in cytokine expression are frequently observed in humans and animals following toxic compound exposure. The importance of cytokine release as an end point for immunotoxicity has been underscored by the cytokine storm phenomenon. The development of reliable in vitro assays, capable of predicting in vivo cytokine release could limit the costs and use of live animals for drug screening processes. In this study, Cynomolgus monkey and human whole blood were stimulated with a variety of agonists (LPS, PMA, PHA and ConA). Following a 24-hour stimulation, various cytokines were measured by Luminex in monkey and human plasma. LPS was injected into monkeys to compare the in vitro data obtained with in vivo cytokine release. Results/conclusion Several cytokines were measured following whole blood in vitro stimulation, especially with LPS. Indeed, IL-1β, IL-6, IL-8, TNF-α and INF-γ increased substantially in plasma following stimulation with LPS. Increases in cytokine levels were also observed following stimulation with PMA and PHA, while ConA showed very little induction of all cytokines measured. When LPS was injected in vivo in monkeys at a dose 10 times lower than the dose used in vitro, a marked increase in the levels of the 2 cytokines measured, IL-6 and TNF-α, was measured 1 hour post LPS injection. These results indicate that in vitro stimulation of whole blood might be an interesting tool for the screening of new compounds susceptible to induce in vivo cytokine release. Alternatively in vitro stimulation of whole blood could also be used to assess the efficacy of immunomodulatory compounds.

PS 1141 EVALUATING THE SECONDARY IMMUNE RESPONSE TO KLH IN CYNOMOLGUS MACAQUES: AN *EX VIVO* APPROACH.

S. H. Cole, P. Gorman, T. T. Kawabata, M. Collinge and S. Casinghino.
Immunotoxicology, Pfizer Global R&D, Groton, CT.

Preclinical toxicology studies with immunomodulatory drugs may require determination of whether an animal can respond to an antigen to which it has previously been exposed, particularly if it is suspected that the drug affects the formation, homeostasis or function of memory T and B lymphocyte subsets. Primary and secondary immune responses can be assessed in vivo in NHP, but where an in vivo approach is either not possible, or undesirable, an ex vivo measure of the secondary immune response is needed. We have developed two in vitro assays for the assessment of a secondary immune response in NHP that utilize Keyhole limpet hemocyanin (KLH) as a tool for ex vivo re-stimulation. Cynomolgus macaques were administered KLH and the primary T-dependent antibody response was assessed in serum by standard ELISA methods for anti-KLH antibody. Three-months following KLH administration peripheral blood mononuclear cells (PBMC) were collected and frozen. PBMC were cultured in the presence or absence of KLH and the proliferative (BrdU incorporation) and antibody response (KLH-specific IgG ELISpot for antibody secreting cells) were measured. To optimize the assays, various concentrations of KLH were evaluated along with the kinetics of the cellular response. KLH re-stimulation elicited robust proliferation after 96 hours when compared to controls in the absence of KLH, or with PBMC isolated from KLH-naïve monkeys stimulated in vitro with KLH. By an indirect ELISpot method, anti-KLH specific IgG secreting cells were detected in PBMC from KLH exposed macaques. In contrast, low numbers of antibody secreting cells were observed with PBMC from KLH-naïve macaques stimulated in vitro with KLH or with PBMC from KLH immunized macaques that were cultured in the absence of KLH. Work described here demonstrates that an ex vivo secondary immune response to KLH in NHP can be performed using either an assessment of proliferation or by anti-KLH-IgG specific immune ELISpot.

PS 1142 DEVELOPMENT OF AN ASSAY TO MEASURE NATURAL KILLER CELL FUNCTION IN CYNOMOLGUS MACAQUES.

S. Haskett, C. Donovan, C. Thiffeault, T. Kawabata and C. Kamperschroer.
Pfizer, Groton, CT.

Natural killer (NK) cells are a subset of lymphocytes that play a pivotal role in the innate immune response to tumors and viral infections. Upon recognition of non-self, activated NK cells produce cytokines and kill the targeted cell. We wanted to develop an assay that measures NK cell function in cynomolgus macaques without cytokine priming. Flow cytometric and plate-based killing assays proved unsuccessful in the absence of IL-2 or other cytokine stimulation due to low signal-to-noise ratios. Here we present the use of an IFNg ELISpot assay to measure NK cell functional activity. PBMCs isolated from cynomolgus macaques were co-cultured with the NK target K562 cell line resulting in the production of IFNg. IFNg secreting cells were observed at frequencies that generally ranged from 100-200 IFNg spots per 1000 NK cells and background levels were negligible. Cell sorting assays confirmed the source of IFNg was in fact due to the activity of NK cells and not T cell activity. Additionally, preliminary evidence suggests that IFNg ELISpot numbers correlate with killing activity. In summary, we developed an assay that enumerates functional NK cells in cynomolgus macaques. In our hands, the assay has a more robust signal than killing assays and is simple to perform. Further, because the assay measures functional activity at the single cell level, it can be used along with flow cytometry endpoints to determine whether deficits in NK cell activity are due to loss of cells or to functional inactivation. Studies to determine sensitivity to immunosuppression are ongoing.

PS 1143 FLOW CYTOMETRY AND REPORTABLE COMBINATIONS: WHEN IS ENOUGH TRULY ENOUGH?

A. R. Mcintyre, T. Salewsky, T. Warren, C. Cornwall, N. Pratt, R. Eyre and H. Tsusaki. *SNBL USA, Ltd., Everett, WA.*

Standard toxicology studies are designed for a specific purpose and, as such have a definite set of parameters evaluated and reported. Part of these evaluations for a typical toxicology study includes immunophenotyping of various cell populations. However, only populations of relevance are typically reported. A vast amount of data are not analyzed, interpreted or reported. Unreported populations may hold evidence of unknown (possibly advantageous) drug effects. Taking these populations into consideration could allow for a deeper understanding of the drug's effects on the immune system. By examining all possible combinations within a panel, extensive data mining can be ormed to isolate populations that may seem inconsequential during protocol development but may play a key role in overall data interpretation and drug assessment. This evaluation was completed on 50 Cynomolgus monkeys, using isolated leukocytes from whole blood. Each sample was stained for CD3, CD4, CD8, CD184, CD62L, CD45RA, CD20, CD40, CD27, CD21, IgD, CD25, CD159a, CD14, CD32, CD64, CD11b, CD16, CD45, CD71 and CD34. From this extensive staining, two gating strategies were evaluated. The first strategy incorporated typically reported combinations such as CD3+, CD3+CD4+, CD3+CD8+, CD3-CD159a+, CD3-CD20+, CD3+CD40+, CD3-CD14+. The second strategy utilized extensive gating; displaying both combinations normally reported as mentioned above and combinations made up of more rare populations that are usually ignored such as CD3-CD8+, CD4+CD8+, CD3+CD4+CD62L+ and CD3+CD8+CD62L+. These strategies were compared and discussed for feasibility and risk management. An extensive number of low prevalence populations were noted that are strong indicators of toxicological effects.

PS 1144 QUALIFCATION OF FLOW CYTOMETRY IMMUNOPHENOTYPING FOR PRE CLINICAL APPLICATIONS IN NON-HUMAN PRIMATES.

T. Salewsky, A. R. Mcintyre, T. Warren, A. Charzynska, N. Clark, R. Eyre and H. Tsusaki. *SNBL USA, Ltd., Everett, WA.*

Flow cytometry immunophenotyping involves identification of cells using light-scattering and the binding of monoclonal antibodies specific for cell phenotypic markers. The enumeration of subsets of cells binding these MAbs is important for the identification of drug toxicity within clinical and pre-clinical evaluations. Published guidelines for human clinical immunophenotyping methods have been described. However accepted guidelines for validating analytical procedures used in flow cytometry laboratories or utilizing NHP models are not nearly as well defined. In light of many papers and the additional CLIA and CAP guidelines, SNBL has instituted a master plan in which to qualify flow cytometric methods.

The purpose of this project was to develop quality control measures, method qualification, and performance guidelines for the enumeration of lymphocyte cell types in the peripheral blood of cynomolgus monkeys using flow cytometry. Here we outline the need for both horizontal and vertical control of instrument and methodology as well as reagents to ensure integrity of all data generated. Though the guidelines established here are widely applicable, the scope of this poster will focus on a wide variety of cell types and subpopulations demonstrating the changes for each and in addition the challenges of multi-site operation of machines to match data sets. Consistent interpretation of flow cytometry data relies on standarization and validation of the instrument, the reagents and the analytical procedure. To achieve this, we assessed single vs. multicolor staining, inter- and intra-assay precision and sample stability for each lymphocyte population. Additionally, QC samples and population summations were performed to assure accuracy. According to the GLP regulations, each laboratory is ultimately responsible for developing methods for its own equipment use and analytical procedures; this poster reviews the validation and quality control of all aspects of the operation of flow cytometry assays in non-human primates

PS 1145 DELAYED-TYPE HYPERSENSITIVITY (DTH) REACTION WITH TETANUS TOXOID (TTX) IN CYNOMOLGUS MONKEYS.

Y. Takahashi, Y. Otsubo, K. Nagatomo, T. Nakamura, T. Sukamoto, K. Fukuzaki and R. Nagata. *Shin Nippon Biomedical Laboratories (SNBL), Ltd., Kagoshima, Japan.*

Purpose: DTH reactions are mentioned in the ICH S8 immunotoxicity guideline as difficult to reproduce and ascertain in monkeys. We investigated sensitization periods and frequencies, and challenge volumes to establish an appropriate design for a DTH reaction test with TTx in cynomolgus monkeys. Method: Three male cynomolgus monkeys per group (total 5 groups) were sensitized one (Groups 1 and 2), two (Group 3), three (Group 4), or four (Group 5) times. They were challenged at three (Groups 1, and 3 to 5) or four (Group 2) weeks after the final sensitization with TTx at 10, 20, and 30 μL/site (Groups 1 and 2; 10 Lf/mL) or at 30 μL/site (Groups 3 to 5; 10, 3, and 1 Lf/mL). The sensitization was performed by injecting TTx (10 Lf/mL) intramuscularly into the femoral region (0.6 mL) and intradermally into the dorsal region (50 μL/site × 12) under anesthesia. The challenge with TTx or physiological saline was made by intradermal injection into the thoracic region (each volume × 3 sites) under anesthesia. Skin reactions (erythema and edema) were evaluated in accordance with the Draize method at approximately 24 and 48 hours after challenge. Result: The mean reaction scores at 24 hours after challenge for TTx (10 Lf/mL) at 30 μL/site in Groups 1, 2, 3, 4 and 5, respectively, were 3.77, 3.06, 2.90, 3.77, and 2.80. The mean reaction scores at 24 hours after challenge for TTx (10 Lf/mL) at 20 and 10 μL/site, respectively, were 3.30 and 2.56 in Group 1 and 3.10 and 2.00 in Group 2. In Groups 3, 4, and 5, respectively, the mean reaction scores at 24 hours after challenge at 30 μL/site were 2.67, 2.97, and 2.10 for 3 Lf/mL TTx, and 1.93, 1.47, and 1.37 for 1 Lf/mL TTx. Mean scores were lower at 48 than 24 hours after challenge. Conclusion: We propose a cynomolgus monkey DTH reaction test with a three-week period and one-time frequency of TTx sensitization, and challenge volume of 30 μL/site for TTx (10 Lf/mL).

PS 1146 IMMUNOTOXICITY ASSESSMENT IN INFANT CYNOMOLGUS MONKEYS BY MEASUREMENT OF HUMORAL IMMUNITY (TDAR) TO KLH AND INNATE IMMUNITY (NATURAL KILLER (NK) CELL ACTIVITY).

F. G. Burleson[1], N. Makori[2], N. Lalayeva[2], G. R. Burleson[1] and H. Tsusaki[2]. *[1]BRT-Burleson Research Technologies, Inc., Morrisville, NC and [2]SNBL, Everett, WA.*

Immunotoxicity assessment of biologics that do not have activity in rodents must be evaluated in non-human primates. Developmental immunotoxicity evaluations are performed to determine if the test article has an immunotoxic effect on the developing immune system. Infant Cynomolgus Monkeys are now routinely used in evaluation of (1) humoral-mediated immunity using a T-dependent antibody response (TDAR) to KLH, and (2) evaluation of innate immunity with NK cell activity. In continuing efforts to standardize methods, enzyme-linked immunoassays were validated to measure KLH-specific IgM and IgG in monkey serum samples. The validation confirmed suitability by evaluation of recovery, dilutional linearity, intra-assay and inter-assay precision and accuracy, and freeze-thaw sample stability. Control background data is obtained from infant Cynomolgus Monkeys starting at 120 days of age by immunization with KLH on DB120 and DB180. Serum is collected prior to the primary KLH immunization on DB120; and 7, 14, 21, and 28 days post immunization to evaluate the primary antibody response to KLH. Serum is also collected on DB180 (prior to KLH boost), and 7, 14, 21, and 30 days post KLH boost to evaluate the secondary antibody response. KLH-specific IgM and IgG antibody concentrations are measured in the serum samples. It is shown that anti-KLH IgG antibody concentrations are elevated 14-30 days post-immunization in individual infants during the primary response. A strong secondary response is observed 7 days after the second KLH immunization. Innate immunity is evaluated by measuring NK cell activity in 100-195 days old infant Cynomolgus Monkeys. PBMC are obtained from whole blood using Ficoll-Hypaque density gradient centrifugation and tested using a minimum of 3 effector:target (E:T) cell ratios. Lysis of K562 target cells is measured by release of 51Cr.

PS 1147 REPRODUCTIVE TOXICOLOGY AND INFANT IMMUNOPHENOTYPING: REFERENCE RANGES FOR T-CELL ASSESSMENT IN INFANTS.

C. Cornwall, A. R. Mcintyre, T. Salewsky, T. Warren, N. Pratt, R. Eyre and H. Tsusaki. *SNBL USA, Ltd., Everett, WA.*

Reproductive toxicology is a necessary part of any drug assessment program intended to evaluate toxicological effects on the mother and infant. Data are available referencing the mature immune systems, which can be used as comparison for data interpretation. However, reference ranges for the developing immune system are not easily accessible due to the rarity of infant animals and the small blood collection volumes possible. Normal ranges are vital to the interpretation and final conclusions made for any data set. Without baseline data conclusions derived may be flawed. Presented is a compiled set of infant reference ranges of T-lymphocytes (T-helper cells, T-cytotoxic cells, as well as rare lymphocyte populations). Samples obtained from 82 cynomolgus monkey neonates (ages ranging between 25 and 35 days) were stained for CD3, CD4 and CD8 cell surface markers. The maximum, minimum, mean, standard deviation and confidence intervals were calculated for the relative percentages of these populations. The mean relative percentage for T-cytotoxic cells (CD3+, CD8+) was 19.0%. The more rare lymphocyte populations (CD3+CD8-, CD3-CD8+, and CD3-CD8-) were 18.7%, 15.0%, and 47.2% respectively. This combined information allows for an accurate assessment of changes in the developing T-lymphocyte population and confidence in data sets with large variation.

PS 1148 DEVELOPMENT OF AN ASSAY TO DETECT CMV AND LCV SPECIFIC T-CELLS TO EVALUATE IMMUNE STATUS IN CYNOMOLOGUS MONKEYS.

P. A. Schneider, L. O'Donnell, T. T. Kawabata and C. Kamperschroer. *Pfizer, Groton, CT.*

The majority of people are chronically infected with the herpesviruses cytomegalovirus (CMV) and Epstein-Barr virus (EBV). These infections are asymptomatic in healthy individuals but can cause serious disease in immune compromised individuals. Cynomolgus macaques harbor both CMV as well as lymphocryptovirus (LCV), the equivalent of human EBV, and immunosuppression can lead to disease similar to that observed in humans. Because chronic CMV, LCV and EBV infections are controlled primarily by T cells, it is useful to measure T cells specific for these viruses in order to assess the risk of viral reactivation. To determine whether candidate drugs affect T cell responses to CMV or LCV, we developed an INF-γ ELISpot assay to enumerate CMV- and LCV-specific T cells in macaques. This is done by incubating cynomologus peripheral blood mononuclear cells with viral lysates and then measuring the number of cells that respond by secreting IFN-γ. Responding cells were confirmed to be T cells and specific for the antigen of interest. Multiple parameters were evaluated in an effort to optimize the response and decrease background. In addition, a 3 month longitudinal study was completed to evaluate variability of the T cell responses to LCV and CMV. Although there is variability in the frequency of responding cells over time, power calculations suggest that it should not preclude using the assay for detecting T cell suppression. Initial in-vitro experiments indicate that cyclosporine reduces the frequency of CMV specific T cells, and future experiments are designed to determine whether this assay is capable of detecting suppression of CMV or LCV-specific T cell responses in immune compromised monkeys. Our ability to monitor LCV and CMV specific T cell function in macaques should greatly improve our ability to assess whether drugs affect immune control of these viral pathogens.

PS 1149 VIRAL LOAD AND LYTIC GENE EXPRESSION ASSAYS TO DETECT REACTIVATION OF LCV IN CYNOMOLGUS MACAQUES.

K. R. Tartaro[1], S. W. Kumpf[2], T. T. Kawabata[1] and C. Kamperschroer[1]. *[1]Immunotoxicology, Pfizer, Groton, CT and [2]DART, Pfizer, Groton, CT.*

Epstein-Barr virus (EBV) is an important pathogen associated with the development of several malignancies in humans. Monitoring of EBV load is commonly used in immunosuppressed transplant patients as a way to assess their risk for developing post-transplant lymphoproliferative disorder (PTLD) or lymphoma.

Lymphocryptovirus (LCV) in macaques is equivalent to Epstein-Barr virus in humans, and LCV can cause disease in immunosuppressed macaques that is similar to that observed in patients. Because LCV can be a serious opportunistic pathogen, it is useful to monitor LCV in macaques during preclinical development, especially when immunomodulatory drugs are being used. Here we present the development of two real time PCR assays to evaluate LCV: a DNA viral load assay and a lytic gene expression assay for BALF-2. To allow for accurate comparisons between samples, both assays include endogenous controls for normalization. The specificity of these assays was confirmed by using peripheral blood mononuclear cells (PBMCs) from monkeys of known LCV status (+/-) as well as by using a positive control LCV+ lymphoblastoid cell line (LCL). LCV was detected in the positive control samples and not detected in samples known to be LCV negative. The virus was also detected in whole blood, PBMCs and nasopharyngeal swabs of cynomologus macaques. Overall, as expected there was a higher level of LCV DNA and BALF-2 gene expression detected in nasopharyngeal samples compared to PMBCs and whole blood. More animals had low or no signal for BALF-2 mRNA when compared to viral load (DNA). These assays appear to be sensitive for detection of LCV and therefore can be used to monitor LCV status. We anticipate that such monitoring will allow us to assess whether LCV is reactivated from latency to lytic phase replication.

PS 1150 IMMUNOGENICITY TESTING OF INFLIXIMAB (REMICADE®) AND ADALIMUMAB (HUMERA®) IN GOTTINGEN MINIPIGS®.

G. J. van Mierlo[1], A. H. Penninks[1], J. Wolthoorn[1], N. H. Cnubben[1], L. Aarden[2] and D. Wouters[2]. [1]*Toxicology and Applied Pharmacology, TNO-Quality of Life, Zeist, Netherlands and* [2]*Sanquin Research, Amsterdam, Netherlands.* Sponsor: R. Woutersen.

As the minipig is currently considered to be a useful alternative species for safety evaluation of (bio)pharmaceuticals we explored their possibilities for immunogenicity testing. In this study the potential immunogenicity of two TNFalpha blockers, Adalimumab and Infliximab both clinically used in chronic inflammation disorders, is evaluated. These pharmaceuticals differ in biological activity in minipigs as Adalimumab is active and Infliximab is inactive. Göttingen Minipigs® (4♂ and 4♀ per group) were treated subcutaneously on days 0, 14, 28, 42 and 56 with low dose (0.1 mg/kg), mid dose (0.1 mg/kg) or high dose (5 mg/kg) Adalumimab or high dose (5 mg/kg/day) Infliximab, followed by a 4 wk recovery period. Clinical signs, injection site reactions, body weight, hematology and the weight and pathology of several organs were used as criteria for disclosing possible adverse effects. Blood samples for anti drug antibody (ADA) determinations and pharmacokinetic (PK) analyses were collected on several days. For PK analysis blood was collected before and on several time points after the first (d0) and the last dosing (d56). At all dose levels of Adalimumab ADA's were present from day 14 onwards. At the lowest dose the most clear time-related increase in ADA's was observed in all animals. At the mid and high dose levels ADA titers were somewhat lower in some animals. PK analysis after the last injection (d56) showed an immune-mediated clearance of Adalimumab in 10/12 minipigs. The Infliximab levels in serum were so high that that they interfered with the ADA analysis (new analysis in progress). Pharmacokinetic analysis showed that after the last injection of Infliximab (d56) no evidence of an immune-mediated clearance was observed with still a very long T1/2 (>14 days). The results obtained will be discussed in connection with those observed in non-human primates and from clinical experience in humans.

PS 1151 IMMUNOGENICITY TESTING OF KINERET® IN GOTTINGEN MINIPIGS®.

A. H. Penninks[1], J. Wolthoorn[2], N. H. Cnubben[1] and G. J. van Mierlo[1]. [1]*Toxicology and Applied Pharmacology, TNO-Quality of Life, Zeist, Netherlands and* [2]*Analytical Research, TNO Quality of Life, Zeist, Netherlands.* Sponsor: R. woutersen.

The aim of this project was to investigate the feasibility of minpigs as alternative species for immunogenicity testing of biopharmaceuticals. In this study the potential immunogenicity of the in minipigs biologically active recombinant human IL-1 receptor antagonist Kineret® was evaluated. Göttingen Minipigs® (3♂ and 3♀ per group) were treated daily subcutaneously for 29 consecutive days with vehicle (water for injection), low dose (0.5 mg/kg/day) or high dose (5 mg/kg/day) Kineret®. Clinical signs, injection site reactions, body weight and the weight and pathology of several organs were used as criteria for disclosing possible adverse effects. Blood samples for anti drug antibody (ADA) determinations and pharmacokinetic (PK) analysis were collected. For PK analysis blood was collected pre dose and several timepoints after the first (day 0) and the last (day 28) dosing. Both low and high dose Kineret®-treated animals showed production of ADA from day 14 onwards. In females ADA production was not dose-related. In males low dose treatment resulted on the average in lower ADA levels than high dose treatment. No remarkable sex-differences were observed in the high dose Kineret® groups for most PK parameters. In the low dose groups on day 28, the Cmax as well as the AUC for Kineret® were higher compared to day 0. As the measured T1/2 of Kineret® was found to be comparable after the first (d0) and the last injection (d28) the ADAs are considered not neutralizing (determination of neutralizing capacity of the ADAs in progress). Tmax was found to be approximately 2 hours and the T1/2 between 1.1 and 2.3 hours. The results obtained in respect to the immunogenicity testing and PK of Kineret® in the minipig were compared with those available in literature for NHP and humans. In summary it was concluded that comparable results were obtained in respect to the immunogenicity and PK of Kineret® in minipigs and non human primates.

PS 1152 ASSESSMENT OF IMMUNE FUNCTION IN CELLS ISOLATED FROM BRONCHOALVELOAR LAVAGE (BAL) FLUID, OBTAINED IN-LIFE IN THE DOG.

V. Leighton, A. Head, G. Hale and S. A. Kirk. *Covance Laboratories Ltd., Harrogate, United Kingdom.*

In-life assessment of the immune system is often restricted to blood measurements. Administration of test item via the inhaled route or when the lung is targeted can mean any changes in blood are secondary and sampling from BAL fluid is more appropriate. Sampling of BAL fluid is often performed as a terminal procedure and sampling during the study requires additional animals. The objective of this study was to determine the feasibility of repeat in-life BAL in the dog and assessment of immune function from the isolated cells. BAL sampling was performed on 2 female, anaesthetised, beagle dogs 1 week apart. Dogs were positioned in left lateral recumbancy and a bronchoscope introduced to the trachea via an adapter fitted to the endotracheal tube. The bronchoscope was advanced until the tip was within a dependent left caudal lobar bronchus. 1.6mL/kg of saline (~37°C) was instilled via the irrigation channel and the fluid re-aspirated. Immunophenotyping was performed on resultant cell suspensions by flow cytometry and phagocytic activity assessed using the Phagotest® kit (Orpegen). Terminal BAL and blood samples were taken on Day 15 in order to provide comparative data. Phenotyping results showed the largest BAL population were CD14+/MHC class II+ (40-70%) followed by CD3+ T cells (10-30%). CD4+ and CD8+ T cells were typically 5-25% and ≥ 5% of total cells, respectively. Limited numbers of cells isolated from each BAL sample meant the Phagotest® had to be adapted to determine the optimum level of phagocytosis possible with the minimum number of cells. Incubation times and cell concentrations were adjusted on each collection day. Optimal results were achieved at 1000 cells/µL when incubated with e.coli for 30 min (15-20% phagocytic cells). Phagocytic activity in blood confirmed the assay performed as expected (40-60% phagocytic cells). In conclusion, in-life sampling of BAL fluid in dogs provides a method for assessing immune function in the lungs.

PS 1153 TRANSIENT KNOCK DOWN OF CHK1 IN BONE MARROW HEMATOPOIETIC PROGENITORS IS LINKED TO BONE MARROW TOXICITY.

B. Jessen and W. Hu. *DSRD, Pfizer, San Diego, CA.*

Check point kinase 1 (Chk1) is required in both intra-S phase and G2/M checkpoints in the cell cycle. It is a protein kinase that plays critical roles in maintenance of genomic stability and transducing DNA damage response. Chk1 is highly expressed in CD4+ and CD8+ T-cells and hematopoietic stem cells. Chk1 deficiency has been shown to block T-cell differentiation. Splenomegaly and severe anemia were observed in a Chk1 haploinsufficiency mouse model. To better understand the role of Chk1 in bone marrow hematopoiesis, we conducted transient Chk1 gene silencing in human bone marrow progenitor cells using Chk1 sequence-specific siRNA and electroporation. Human bone marrow mononuclear cells and CD34+ stem cells were cultured in HPGM media, and induced to undergo differentiation and proliferation in the presence of appropriate amount of hematopoietic cytokines. The electroporation procedure with a negative control siRNA was shown to have minimal impact on cell viability as compared to a non-electroporated control. At 48hr post electroporation, more than 70% knock down of Chk1 was confirmed at the mRNA level using real-time RT-PCR and at the protein level using immunoblotting. On day 6, hematopoietic progenitor cells electroporated with Chk1 specific siRNA resulted in more than 60% reduction in cell count as compared to the scrambled control. The inhibition of hematopoietic progenitor differentiation and proliferation by Chk1 knock down was confirmed using a colony unit forming assay, where reduced number of erythroid and granulocyte colonies was observed with Chk1 siRNA treatment. We established in this study an efficient method to knock down a gene of interest in hard-to-transfect hematopoietic stem cells and studied the association with bone marrow toxicity. Furthermore, our results support a direct role of Chk1 in maintaining normal hematopoiesis in the

bone marrow. Since Chk1 is an attractive Oncology target, Chk1 inhibitors currently under development should be examined closely for potential bone marrow toxicity.

PS 1154 RADIATION AND SENESCENCE: DECELERATION OF CELL-CYCLE IN PRIMITIVE HEMOPOIETIC PROGENITORS (CFU-S13) WAS ONLY SIGNIFICANT PARAMETER DURING AGING, WHICH WAS REACTIVELY ACCELERATED AFTER 2GY WHOLE-BODY IRRADIATION.

Y. Hirabayashi[1], I. Tsuboi[2], K. Sekita[1], J. Kanno[1], Y. Kusunoki[3] and T. Inoue[1, 2, 3]. *[1]NCBCR, NIHS, Tokyo, Japan, [2]School of Medicine, Nihon University, Tokyo, Japan and [3]RERF, Hiroshima, Japan.*

Toxicology is a function of xenobiotic responses, and aging is a function of xenobiotic responses during lifetime; thus, "time" during life is an essential toxicologic parameter for living creatures. Effect of ionizing radiation during lifetime is thus formulated by a function of simultaneous equations over the effects of "lifetime" and "radiation". Owing to the mission of NIAID, the study was conducted to evaluate a possible additive/ synergic effect of radiation exposure during aging in cell cycling parameters of the hematopoietic stem (HSC) /progenitor cell (PGC) compartment (Contract No. HHSN 272 200 900 059C). Kinetics of cell cycles in HSC/PGC (GM, mature PGC = CFU-GM; S9, less mature PGC = CFU-S9; and S13, immature PGC = CFU-S13) were evaluated by the BUUV assay (The HSC/PGC-specific cell kinetics, Hirabayashi Y. et al. Exp Biol Med 227:474-9, 2002; Sf/T, S phase fract./unit time; DT, Doubl. time; Cf, cycling fract.). During the steady state, old mice (Om; 23 mos.) showed, as compared with young mice (Ym; 3 mos.), an increased to a decreased rate of Sf/T in GM ~ S13; a decelerated to an accelerated DT from GM ~ S13; and an increased to a decreased Cfs from GM ~ S13, respectively. When both Ym and Om were irradiated with 2Gy and given a recovery time for 4 weeks, each parameter of both groups showed; the rates of Sf/T were increased in all PGC from GM ~ S13 in Ym, whereas in Om, rates of Sf/T were even to decreased from GM ~ S13; DTs in all PGC were decelerated in both groups, except accelerated in Om-S13; and similarly, Cfs were decreased in GM and S9 in both Ym and Om, whereas solely increased in S13 in both groups. In conclusion, changes in the effects of aging and radiation exposure for HSC/PGC were significant only in the primitive PGC (CFU-S13), in which cell cycle was depressed during aging, and was contrarily accelerated reactively after radiation only in the primitive PGC.

PS 1155 CADMIUM-INDUCED TRANSFORMATION OF HUMAN STEM CELLS.

E. Tokar and M. Waalkes. *National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Cadmium (Cd) is a common environmental contaminant linked to human prostate cancer. Strong evidence indicates cancer is a stem cell (SC)-based disease, due, in part, to the inherent resistance and indefinite proliferative potential of SCs, allowing accumulation of sufficient oncogenic "hits" to drive neoplasia. We have recently observed SCs possess an innate resistance and hyperadaptability to arsenic, manifesting as a cancer SC (CSC) overproduction unique to arsenic-induced malignant transformation but not seen with Cd-induced transformation. To determine the reasons for this difference between these carcinogenic inorganics and CSC dynamics, a human prostate epithelial SC line, WPE-stem, and its mature parental cell line, RWPE-1, were chronically exposed to low-level (10 μM) Cd for up to 10 weeks. Early exposure was highly toxic to SCs (90% toxicity at 1 week) but, remarkably, not to mature epithelial cells. This is unusual given that SCs are typically resistant to toxicants. The SCs rapidly recovered and were growing at a rate similar to mature epithelial cells or control SCs by 4 weeks. Thus, it appears Cd causes an early "bottleneck" effect specifically on SCs. Based on secreted MMP-9 activity, a well-established marker for malignant transformation, the SCs were potentially transformed by Cd in only 4 weeks (1500% increase in MMP-9) while parental cells required 10 weeks of Cd exposure before indications of transformation occurred (400% increase in MMP-9). This accelerated transformation of SCs compared to their mature counterparts is similar to that seen with arsenic in the same cells, indicating the stage of differentiation may be a key factor in time to acquired malignant phenotype with both inorganics. Together, these data show that, similar to arsenic, Cd can induce an acquired cancer phenotype in SCs and does so much quicker than in mature cells. However, in direct contrast to arsenic, Cd selectively kills SCs in early stages of transformation. This could account for the quantitative difference in CSCs between these two inorganics upon acquired malignant phenotype.

PS 1156 GROWTH HORMONE *VS.* PEGYLATED GH LIPOATROPHY IN HUMAN STEM CELL DERIVED ADIPOCYTES CHRISTOPHER DONAHUE[1], MARK THIEDE [1], SUSAN MARTIN[2],, JANIS VAJDOS [1], AND PHILLIP M. BARTHOLOMEW[3] GENETICALLY MODIFIED MODELS CENTER OF EMPHASIS[1], BIOLOGICS PHARM R&D[2], AND DRUG SAFETY R&D[3] PFIZER R&D, GROTON, CT 06340.

P. M. Bartholomew[1], C. Donahue[2], M. Thiede[2], S. Martin[3] and J. Vajdos[2]. *[1]Drug Safety R&D, Pfizer Inc., Groton, CT, [2]Genetically Modified Models, Pfizer Inc., Groton, CT and [3]Pharm R&D, Pfizer Inc., Groton, CT.*

Injection site lipoatrophy occurs at a low incidence with some injectable drugs (e.g. recombinant human growth hormone (rhGH) and insulin). In vitro, GH inhibits lipid accumulation and stimulates lipolysis in adipocytes, a pharmacological action consistent with lipoatrophy. Since rhGH therapy requires daily injections due to a 2-3 hr half-life, a polyethylene glycol-GH (PEG–GH) was created to provide a weekly dosing option. While 3 month animal toxicology studies with PEG-GH showed no evidence of lipoatrophy, it was observed at the injection site clinically. To address the mechanism of lipoatrophy, we used a dynamic adipocyte culture differentiated from human mesenchymal stem cells (hMSCs). We explored the concentration-dependent action of rhGH on lipid accumulation during adipocyte formation (Days 4-19) and maintenance (Days 19-33) as well as lipolysis of mature adipocytes. We found GH receptor mRNA to be induced (up to 25 fold) during differentiation and expressed throughout the 33 day culture. Addition of PEG-GH (0.003-30 μg/ml), but not PEG, during adipocyte formation or maintenance reduced lipid content concentration dependently, 12-36% on Day 19, 5-25% on Day 33. In contrast, all concentrations (0.001-10 μg/ml) of rhGH reduced lipid content (~22%) at both time points. Lipolysis measured on Day 19 by glycerol release increased in a concentration dependent manner after 4 hour incubations with rhGH (0.001-10 μg/ml). PEG-GH stimulated lipolysis non-dose dependently and to a lesser degree than GH. Exposure of GH receptor-expressing human adipocytes to GH receptor agonist results in lipid loss consistent with clinical lipoatrophy. Thus this in vitro model appears suitable for evaluating the lipoatrophy potential of new therapies.

PS 1157 DIOXIN, A POTENT LIGAND OF THE ARYL HYDROCARBON RECEPTOR, ALTERS MIGRATION OF MURINE HEMATOPOIETIC STEM CELLS.

F. L. Casado-Pena, K. P. Singh and T. A. Gasiewicz. *Environmental Medicine, University of Rochester, Rochester, NY.*

Human exposure to high levels of dioxins has been associated with increased incidence of leukemia and lymphoma. A plausible mechanism to support these claims has not been defined. Previously, we reported that the exposure of mice to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) leads to increased relative numbers of phenotypically-defined cell populations in bone marrow, and compromised reconstitution of bone marrow after transplantation into irradiated host animals without changing the absolute numbers of HSCs with short- and long-term repopulation. AhR's antagonists have been reported to promote the expansion of human hematopoietic stem cells (HSC) in vitro, and HSCs from AhR-/- mice are more proliferative. Here, we tested the hypothesis that TCDD changes the expression of genes involved in the migratory behavior of HSCs to the bone marrow. In vivo and in vitro migration of TCDD-treated (30 μg/kg, 7 days) HSC-enriched populations of cells were altered by a mechanism involving phenotypic changes in CXCR4, a receptor for a bone marrow chemotactic factor, and CD44, a receptor for molecules present in the extra-cellular matrix of the bone marrow. Gene expression profiles were studied at 6 and 12 hours after treatment and are consistent with a TCDD effect on cellular trafficking of phenotypically-enriched HSCs. Our data support a genomic-mechanism for TCDD to alter the phenotype, function and migration of HSCs. Supported by NIH Grants ES04862, ES01247, ES016606.

PS 1158 ARYL HYDROCARBON RECEPTOR AGONIST DIOXIN PRODUCES ALTERATIONS IN EARLY HEMATOPOIESIS IN MOUSE FETAL LIVER.

K. P. Singh, F. L. Casado and T. A. Gasiewicz. *Environmental Medicine, University of Rochester Medical Center, Rochester, NY.*

The aryl hydrocarbon receptor (AhR) is a highly conserved, developmentally-regulated and ligand-dependent transcription factor in the basic helix-loop-helix superfamily of DNA-binding proteins. The 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; Dioxin) is the most potent ligand for AhR known, and it is used to study

the AhR. Despite extensive research, the normal function of the AhR is unknown. Recently, we have found that TCDD treatment to the mice can produce altered number and function of hematopoietic stem/progenitor cells (HSPCs). The physiological role of the AhR in HSC function has been further confirmed by us using AhR-KO mice and by others in human HSC. Though hematopoiesis starts early in developing embryonic stage at E7 day but definitive adult-like hematopoiesis starts in fetal liver and then progresses to spleen and bone marrow. This study was undertaken to determine the effect of TCDD on early hematopoiesis. C57BL/6 mice were exposed to TCDD (5 and 30 μg/kg body wt.) at E13 day pregnancy and the studies were taken using fetal liver isolated from E18 day. The TCDD exposed fetal livers have shown significant increase in weight and total cell count. Increases in the number of phenotypically-defined HSPCs were observed and B- and myeloid lineage cells were also increased. Fetal liver cells from TCDD exposed group have lower functionally-defined progenitor and lineage cells determined by colony forming assays. Fetal liver cells exposed to 5 nM TCDD in vitro have also exhibited lower numbers of these colony forming cells. HSPCs isolated from fetal livers of TCDD exposed mice have also demonstrated lower proliferation ability in the presence of EPO and SCF+EPO. Taken together our data shows that TCDD produces significant alterations in early hamatopoiesis which confirmed our earlier hypothesis that AhR has a role in physiological function of HSPCs. (Funded by NIH Grants ES04862, ES016606, ES01247).

PS 1159 INVOLVEMENT OF AMPK IN SUNITINIB MEDIATED CARDIOTOXICITY IN HUMAN STEM CELL DERIVED CARDIOMYOCYTES.

J. D. Cohen, S. Kameoka, J. Babiarz, T. Weiser, T. Singer and K. Kolaja. *Non-Clinical Safety, Hoffmann-La Roche, Nutley, NJ.*

Sunitinib, an oral tyrosine kinase inhibitor used to treat advanced renal cell carcinoma and gastrointestinal stroma tumor, is associated with cardiac toxicity. The mechanism of cardiotoxicity is not fully known, but potential role of direct inhibition of specific kinases including AMPK has been proposed. We utilized an induced pluripotent stem cell-derived model of human cardiomyocytes (iCell Cardiomyocytes, Cellular Dynamics) to investigate the mechanism of sunitinib-mediated cardiotoxicity. The iCell cardiomyocytes are terminally differentiated, express adult cardiomyocyte markers and have the electrophysiological and contractile properties of cardiomyocytes. Sunitinib treatment of iCell cardiomyocytes resulted in a dose dependent change in ATP depletion, caspase 3/7 cleavage, lactate dehydrogenase (LDH) release, glutathione (GSH) depletion, and cell viability. To examine the significance of off-target inhibition of AMPK in sunitinib cardiotoxicity in a human model, iCells were pretreated with AMPK activators AICAR or metformin. Stimulation of p-AMPK-S79 and its downstream substrate p-ACC-T172 was observed by Western following metformin or AICAR treatment in iCell cardiomyocytes. Slight attenuation of sunitinib cardiotoxicity was observed following pretreatment of metformin as measured by decreased LDH release, GSH depletion, and cell death. Similarly, AICAR pretreatment resulted in a statistically significant reduction of LDH release, GSH depletion, caspase 3/7 cleavage, ATP depletion, and cell death. Our findings suggest that AMPK may play a role in sunitinib mediated cardiotoxicity and more broadly, iCell cardiomyocytes are a useful model for investigating mechanism of cardiotoxicity.

PS 1160 HYPOTHERMOSOL—DERISKING A VEHICLE.

C. Potter[1], K. Hedley[1], M. Sharpe[1], P. Cleall[1], A. Raber[2], W. Vant Hof[2], R. Deans[2] and A. Mathew[3]. [1]*Pfizer, Sandwich, Kent, United Kingdom,* [2]*AtherSys, Cleveland, OH and* [3]*Biolife Solutions, Bothell, WA.*

HypoThermosol® FRS (HTS; Biolife Solutions Inc.) an optimized media for improved and extended preservation of cells, tissues and organs is increasingly used in cell therapy applications which require prolonged stability. A single dose intravenous (IV) study in Wistar Han rats using HTS as the vehicle resulted in oedema in all animals of all treatment groups immediately post dose, that persisted <8 hours. At necropsy there were no clinical pathology, histology or pathology findings. It was hypothesised that this reaction was linked to a componenet of HTS, most likely Dextran-40 or an antioxidant (a water-soluble vitamin E analogue). A previous study in C57Bl/6 mice (BioLife, FHCRC) had not shown this effect. To determine if these effects were species specific and identify the responsible component a series of studies were implemented. A single dose IV study of HTS was administered to Balb/c and C57Bl/6 mice, Wistar Han and SD rats, along with an exploratory non-terminal toxicity study in Cyno Monkeys. In addition a single dose IV study of HTS and its components (Dextran-40, antioxidant and Dextran-40/antioxidant) was performed in Wistar Han rats. All doses were equivalent on a v/v basis in blood to that proposed for administration to humans and given by slow bolus injection. Standard clinical chemistry and haematology were performed along with histamine and cytokine (IL-1β, IL-4, IL-5, IL-13, KC/GRO, IFN-γ) analysis. Animals showing clinical signs of oedema were necropsied as soon as possible. Following HTS administration no reactions were observed in mice or monkey. Oedema was observed in rats following administration of HTS, Dextran 40, or Dextran 40/antioxidant. Histamine and cytokine analysis reinforced these findings. Literature reports indicate that rats are hypersensitive to Dextran-40. Taken together, the results from the completed studies in mice, rats and the cynomolgus monkey indicate that the finding in rats are species-specific and were caused by an acute hypersensitivity reaction to the dextran-40 component of the HTS vehicle.

PS 1161 CARDIAC TROPONIN I AS A MARKER OF CARDIOTOXICITY IN HUMAN-INDUCED PLURIPOTENT STEM CELL CARDIOMYOCYTES.

R. Abrams[1], M. Dunn[1], D. Coluccio[1], G. Hirkaler[1], J. Babiarz[1], R. Nicklaus[1], W. Geng[1], I. Mikaelian[1], T. Singer[2] and K. Kolaja[1]. [1]*Nonclinical Safety, Roche, Nutley, NJ and* [2]*Nonclinical Safety, Roche, Basel, Switzerland.*

Background: Advances in the generation and production of human induced pluripotent stem (hIPS) cell cardiomyocytes allow for investigation of their utility in screening compounds for cardiotoxicity (CT) in vitro. Cardiac troponin I (cTnI) release is well established as a marker of myocardial damage. We assessed the hypothesis that hIPS cell cardiomyocytes release detectable levels of cTnI in response to chemotherapeutic agents. Methods and Results: hIPS cell cardiomyocytes were treated with doxorubicin (D), sunitinib (S), imatinib (I), or control for 24 or 48 hours. cTnI concentrations in the media and intracellular ATP levels were assayed. A concentration- and duration-dependent relationship was established between cTnI and ATP levels. Release of cTnI after treatment for 24 hours was observed in the two highest doses of D and S. After 48 hours of D, cTnI concentrations ranged from 33-700% above baseline and corresponded with an 8-23% decline in ATP. 48 hours of treatment with the lower doses of S or I produced a concomitant elevation of cTnI (200-400%) and ATP (16-28%). ATP decreased and cTnI increased (98 and 2,667%, respectively) only for the highest dose of S. Conclusion: Our results demonstrate that cTnI release from hIPS cells occurs in a concentration and duration dependent manner, simultaneous with a decline of ATP. Dosing for 48 hours with S or I induced a divergent cellular response at the lower concentrations possibly due to compound specific survival responses as evidenced by an initial increase of both ATP and cTnI. As indicated by a decrease of ATP and elevation of cTnI, CT was observed only at higher concentrations after 48 hours of S dosing. In conclusion, we have established a dose response relationship between ATP and cTnI in hIPS cell cardiomyocytes given chemotherapeutic agents. Our work uniquely identifies the utility of hIPS cell cardiomyocytes and measurements of cTnI as a potential biomarker for further validation in the screening of compounds for CT.

PS 1162 *IN VITRO* TOXICITY OF (THIO)ARSENATES FOR HUMAN UROTHELIAL CELLS (UROTSA) AND HEPATOCYTES (HEPG2).

S. Rabieh[1], R. Lohmayer[1], S. Hinrichsen[1], E. Dopp[2] and B. Planer–Friedrich[1]. [1]*Environmental Geochemistry, University of Bayreuth, Bayreuth, Germany and* [2]*Institute of Hygiene and Occupational Medicine, University Hospital Essen, Essen, Germany.*

Inorganic arsenic is a known human carcinogen, inducing tumours to skin, bladder, and liver. Many studies have been performed on the cytotoxicity of oxyarsenicals but little is known about the toxicity of thioarsenicals. Inorganic thioarsenicals are the predominant arsenic species in sulfidic environments and we postulate that they can also form *in vivo*, similar to organic thioarsenicals which were found in human and animal urine. The goal of this project was to investigate the *in vitro* toxicity of mono– and trithioarsenate compared to arsenite, arsenate, mono– and dimethylarsenate, and to determine additive, synergistic, or antagonistic effects of mixtures of different arsenic species. UROtsa and HepG2 cell lines were chosen because the liver is the primary site of arsenic metabolism *in vivo* and the bladder is a main target organ for arsenic carcinogenicity. Cells were exposed to arsenic concentrations from 0.1 μM to 10 mM for 1, 24, 48, and 72 h, and *in vitro* cytotoxicity was determined using the MTT test. None of the tested arsenicals were cytotoxic after an exposure time of 1 hour. After 24 hours, LC_{50} values for UROtsa cells showed the following order of toxicity: Arsenite⟩ arsenate⟩ monothioarsenate⟩ trithioarsenate⟩ dimethylarsenate⟩ monomethylarsenate. The same order of decreasing toxicity was observed for HepG2, apart from monothioarsenate which was more toxic than arsenate. After 48 and 72 h, toxicity increased for all arsenicals in both cell lines. The effect was especially noticeable for monothioarsenate which was almost as toxic as arsenite after 72 hour exposure. Mixtures of different arsenicals showed additive toxic effects. Overall, formation of thioarsenicals seems to be, at least on short-term exposure, a detoxification mechanism with decreasing toxic effects with an increasing number of thio-groups.

PS 1163 THE MRNA AND MIRNA PROFILE OF HUMAN IPS-CELLS DIFFERENTIATING TO CARDIOMYOCYTES.

J. Babiarz[1], M. Ravone[2], B. Anson[3], C. Kendrick-Parker[3], E. Nuywasir[3], T. Weiser[2], T. Singer[2], E. Chiao[1], U. Certa[2] and K. Kolaja[1]. *[1]Nonclinical Safety, F. Hoffman-La Roche, Nutley, NJ, [2]Nonclinical Safety, F. Hoffman-La Roche, Basel, Switzerland and [3]Cellular Dynamics International, Madison, WI.*

Cardiac-related side-effects are one of the primary causes of pharmaceutical compound attrition and recall, yet in vitro models rely on either primary rodent cells, or cultured primary human cardiomyocytes that have dedifferentiated, proliferate, and have lost their electrophysiological characteristics. To overcome these insufficiencies, we turned to human induced Pluripotent Stem Cell (hiPSC)-derived cardiomyocytes, which are human, do not proliferate, and have the full complement of ion channels compared to the adult human heart. To gain a more complete picture of this important model, we profiled the mRNA and miRNA content across the hiPS to cardiomyocyte differentiation protocol. Gene Ontology (GO) analysis of different expression classes revealed 3 primary categories: pluripotent-specific, switch genes, and cardiomyocyte-specific. We have found that cardiomyocytes differentiated from pluripotent stem cells are more closely related to intact adult heart gene expression, more so than primary culture cardiomyocytes. Hierarchical clustering of the mRNA data revealed that once cells have differentiated into cardiomyocytes, their transcriptome is stable. Hierarchical clustering of miRNAs showed that, like the transcriptome, the miRNA content does not largely change following cardiomyocyte specification. Analysis of cardiomyocyte-specific miRNAs (miR-1, miR-133a/b, and miR-208a/b) revealed the expected pattern of miRNA induction occur concomitant with cardiomyocyte specification. Finally, we examined the effect of extended culture on cardiomyocyte maturation and indicate that with increasing time in culture, hiPSC cardiomyocytes progressively mature towards an adult heart expression pattern. Taken together, these results reveal a hiPSC cardiomyocytes are a maturing cell population with an expression profile similar to adult heart cells.

PS 1164 USE OF INDUCED PLURIPOTENT CELL-DERIVED CARDIOMYOCYTES TO PREDICT AND INVESTIGATE DRUG INDUCED CARDIAC INJURY.

S. Kameoka[1], J. Babiarz[1], B. Anson[3], C. Kendrick-Parker[3], E. Nuwaysir[3], T. Weiser[2], T. Singer[2], E. Chiao[1] and K. Kolaja[1]. *[1]Nonclinical Safety, F. Hoffman-La Roche, Nutley, NJ, [2]Nonclinical Safety, F. Hoffman-La Roche, Basel, Switzerland and [3]Cellular Dynamics International, Madison, WI.*

Early stage, in vitro models for examining cardiotoxicity are currently lacking, with only surrogates available. To overcome this hurdle, an in vitro model of cardiotoxicity assessment was developed using human induced Pluripotent Stem Cell (hiPS)-derived cardiomyocytes with a panel of compounds with known toxicological sequelae. To determine the best point in the differentiation time course for assaying cardiotoxicity, we determined cell viability IC50 values for Gleevec, Iressa, Tykerb, Doxorubicin, and Sutent at four different differentiation endpoints. Once the appropriate differentiation time point was determined, we were able to correctly differentiate compounds that were directly cardiotoxic versus non-cardiotoxic. However, compounds that elicit cardiotoxicity through indirect means, such as hypertrophy or inhibition of ion channels were missed. To create a high-throughput assay with low compound usage, we examined a subset of our extensive dataset generated in 96 well format in 384 well format. These data correlated well, allowing for multiple tests to be performed in a single assay run. Finally, we embarked upon a careful mechanistic study using the hiPS-derived cardiomyocytes, where we determined the IC50 value for cell viablitiy, GSH, and Caspase 3/7 activation for 6 compounds: Gleevec, Sutent, Nexavar, Zactima, Doxorubicin, and Angiotensin II. Taken together, our study reveals that hiPS-derived cardiomyocytes are a powerful platform for identifying direct cardiotoxicity and studying the underlying mechanisms.

PS 1165 FAST IMPEDANCE ANALYSIS IN HIPSC-DERIVED CARDIOMYOCYTES FOR EARLY IDENTIFICATION OF CARDIAC DRUG LIABILITY.

L. Guo[1], R. Abramas[1], T. Singer[2], T. Weiser[1, 2], J. Babiarz[1], E. Chiao[1] and K. Kolaja[1]. *[1]NCS Early & Investigative Safety, F. Hoffmann-La Roche, Inc., Nutley, NJ and [2]Nonclinical Safety, F. Hoffmann–La Roche Ltd., Basel, Switzerland.*

Cardiac liability has been the primary cause of safety-based drug attrition in the past decade. This was attributed traditionally to cytotoxicity of chemotherapy reagents, and more recently, to arrhythmogensis of drugs from largely diverse therapeutic classes. Early identification of both cardiotoxic and proarrhythmic liability becomes imperative for drug attrition reduction in the later stages of development. This study evaluated the application of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes in xCELLigence RTCA Cardio system, a new impedance analysis tool with a high-sampling rate suitable to monitor cardiac viability and function, in a 96-well format. The sensitivity, specificity and predictive value of this cellular model were validated with over 30 reference compounds which included well-defined cardioactive compounds including cardiotoxicants, ion channel/transporter blockers, receptor modulators and myosin inhibitors, as well as cardiac inert compounds. All compounds were assessed relative to concentrations necessary to achieve a therapeutic exposure. The results demonstrated that 1) impedance measurement was a suitable parameter to interrogate the physical properties of cardiac contraction; 2) the beating amplitude was a robust indicator of cardiac contractility modulation; and more importantly 3) the beating pattern analysis was powerful enough to reveal the proarrhythmic propensity of a compound, as the overt arrhythmic beats were reproducibly elicited by arrhythmogenic compounds at the concentrations relevant to that inducing severe arrhythmia, e.g. torsade de pointes, in either the clinical or pre-clinical settings. Thus, this novel model of fast impedance analysis in hiPSC-derived cardiomyocytes could be a valuable platform for simultaneous identification of cardiotoxicity, contractility alteration and proarrhythmic risk of drug candidates at the early stage of drug discovery and development.

PS 1166 VARDENAFIL INCREASES THE BEAT RATE OF HUMAN CARDIOMYOCYTES *VIA* POTENTIATION OF HCN4 CHANNEL CURRENTS.

A. Bruening-Wright, Y. A. Kuryshev, J. W. Kramer, A. MacCormack, T. Yang, W. Pei and A. M. Brown. *ChanTest Corp., Cleveland, OH.*

Vardenafil (brand name Levitra®) is a phosphodiesterase (PDE) inhibitor used to treat erectile dysfunction in men by increasing blood flow during sexual stimulation, thereby enhancing penile rigidity. Vardenafil's vasodilatory action, however, is not limited to the penis, and systemic side effects, including reduction in systolic and diastolic blood pressure and increased heart rate, have been observed in clinical trials. The vardenafil-induced increase in heart rate is generally believed to be a compensatory autonomic response to the drop in blood pressure. However, electrophysiological profiling of a panel of cloned cardiac ion channels indicated that vardenafil may inhibit voltage gated potassium (Kv1.5) and calcium ion channels (Cav1.2) and increase hyperpolarization-activated, cyclic nucleotide-gated ion channel (HCN4 channel) currents. Using manual perforated-patch clamp of single, isolated, stem cell-derived human cardiomyocytes (SC-hCMs), and cloned HCN4 channels, we demonstrate that vardenafil directly potentiates HCN4 current and increases the contraction rate of SC-hCMs in the absence of autonomic input. Therefore, vardenafil may act directly on cardiac tissue to induce off-target effects.

PS 1167 GENERATION AND VALIDATION OF CELL BASED REPORTER SYSTEM TO MONITOR EPIGENETIC CHANGES IN HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS.

I. Gurevich, W. Krueger and T. P. Rasmussen. *Pharmaceutical Sciences, University of Connecticut, Storrs, CT.*

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) are emerging as an important model system in many aspects of toxicology. Their capacity to differentiate into any cell type in the body makes them particularly useful in early safety assessment in the drug discovery process. Unfortunately, hESCs and iPSCs have been shown to undergo spontaneous epigenetic changes during their *in vitro* culture which limits their utility in toxicologic and other applications. These changes often result in no apparent alteration in cell morphology or pluripotency making their detection particularly difficult. We therefore undertook the design and validation of a cell based system that would enable the monitoring of these epigenetic changes. The system is based on the expression of fluorescent and luminescent reporter genes driven by promoters of genes known to respond to cellular epigenetic states. Reporters are delivered by lentiviral infection allowing for stable integration into the cell genome, which facilitates monitoring of epigenetic changes. Puromycin selection and multiplicity of infection analyses are used to isolate cells carrying the complete set of reporters. Lentiviral delivery coupled with the use of iPS reprogramming will enable any cell expressing the reporters to be directed to differentiate into any cell type, e.g., hepatocytes or cardiomyocytes, providing a unique opportunity to assess toxicant response in human cells. Beyond that, this reporter system would serve as a platform for monitoring spontaneous epigenetic changes in the hESCs and iPSCs and for high throughput screening of compounds and culture methods to aid in stabilization of these cells in pristine state and prevention of epigenetic drift. The rapid detection of alteration in pluripotency and epigenetic changes in hESCs and iPSCs will greatly improve the reproducibility of the experimental results, which will improve their utility in toxicity testing and safety screening.

PS 1168 DIFFERENTIAL PAH EFFECTS MEDIATED BY CYP1A1 AND CYP1B1 IN THE BONE MARROW SPECIFICALLY TARGET HEMATOPOIETIC PROGENITORS.

M. L. Larsen[1], A. U. N'Jai[3], C. J. Czuprynski[3, 2] and C. R. Jefcoate[1, 2]. [1]*Pharmacology, University of Wisconsin, Madison, WI,* [2]*Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI and* [3]*School of Veterinary Medicine, University of Wisconsin, Madison, WI.*

The polycyclic aromatic hydrocarbons (PAHs), benzo(a)pyrene (BP) and 7,12-dimethylbenzanthracene (DMBA), produce very different effects in a Min-mouse cancer model and differential suppression of hematopoietic progenitors in mouse bone marrow (BM). In each model, DMBA is much more active than BP and the effects are dependent on Cyp1b1. Colony forming unit assays show that DMBA suppresses lymphoid progenitor activity within 6 h. BP is much less effective, due to an Ah receptor (AhR)-mediated recovery process. Microarray analyses show few DMBA responses, but numerous BP responses (25 versus 600). Nine gene responses, which were similar for each PAH and for TCDD, are attributed to direct AhR stimulation. These include Cyp1a1, Cyp1b1, and AhRR. Many BP responses, like the recovery process, were AhR dependent, but resistant to DMBA. The time dependence of BP-responses demonstrated an inflammatory group that responded within 6 h (chemokines, cytokines, COX-2) and decayed within 24 h, and a second group (Nqo1 muGSTs), which showed the inverse response. Gene expression changes in BM of Cyp1b1-/- mice demonstrated the substantial altered expression of many developmental regulators. Remarkably, inflammatory gene responses to DMBA, which were absent in WT mice, were increased to BP levels. Thus, removal of BM Cyp1b1 metabolism appears to enhance local DMBA responses. In Cyp1a1-/- mice, many BP-inflammatory responses declined and a cluster of other gene responses increased. We hypothesize that Cyp1a1 generates BP quinones in BM that initiate selective oxidative stress responses that contribute to protection, while endogenous Cyp1b1 metabolites control developmental processes, consistent with a role in embryogenesis.

PS 1169 IMMUNOMODULATORY RESPONSE OF MOUSE BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS (MBMSCS) TO STIMULATION WITH LIPOPOLYSACCHARIDES (LPS).

N. V. Gorbunov, B. R. Garrison, G. Ledney and J. G. Kiang. *Radiation Combined Injury Program, Armed Forces Radiobiology Research Institute, Bethesda, MD.*

Mortality induced by gram-negative bacterial sepsis remains a serious problem of complicated injury due to penetrating wounds, burns, blunt trauma, radiation, chemical exposures, or combinations thereof. A promising therapeutic tool for management of trauma and sepsis is transfusion with bone marrow-derived mesenchymal stem cells (BMSCs) that exhibit immunomodulatory capacity. However, the molecular mechanisms underlying BMSC action in septic conditions are poorly understood. Thus, this report focuses on in vitro investigations of the response of mouse BMSCs (mBMSCs) to stimulation with products of gram-negative bacteria, LPS. mBMSCs were obtained from the femur bone marrow of B6D2F1/J female mice. The cells were expanded and cultivated in hypoxic conditions for 28 days in MESENCULT medium. The cell phenotype, proliferative and colony-forming activity were analyzed with flow cytometry and immunofluorescence imaging; the cells were identified by BMSC-positive markers: CD44, STRO1, SCA1. mBMSCs were exposed to 0.05 - 2.5 μg/ml LPS for 1-3 h and analyzed at different timepoints. LPS-induced gene and protein expression of immunomodulatory effectors (IL-1, IL-6, IL-8, and iNOS) were determined by qRT-PCR and immunoblotting techniques. Nuclear translocation of p65-NFkB and nitric oxide (NO) production in the cells was assessed with fluorescent imaging. Treatment of mBMSCs with LPS resulted in a substantial increase in gene expression of IL-1α, IL-1β, and iNOS that occurred in a dose- and time-dependent manner. At 24 h following exposure to 0.5 μg/ml LPS, expression of NFkB, IL-1β, and iNOS proteins, and NO production in the cells was increased 2-, 12-, 10-, and 14-fold, respectively. These data are consistent with the idea that LPS-induced alterations in mBMSCs are mediated by the Toll-like receptor 4/NFkB axes that, in turn, modultate the host adaptive responses during septic conditions. (Supported by NIH/NIAID YI-AI-5045-04).

PS 1170 FINE PARTICULATE MATTER ($PM_{2.5}$) EXPOSURE AFFECTS CIRCULATING AND BONE MARROW ENDOTHELIAL PROGENITOR CELLS.

P. Haberzettl, J. Lee, D. Duggineni, J. McCracken, A. Bhatnagar and D. J. Conklin. *Diabetes and Obesity Center, University of Louisville, Louisville, KY.*

Inhalation of fine particulate matter ($PM_{2.5}$) induces endothelial dysfunction and increases the risk for cardiovascular disease and mortality. Endothelial progenitor cells (EPC) contribute to endothelium health and we reported that exposure to ambient or concentrated ambient $PM_{2.5}$ (CAPs) decreases the number of circulating EPC in humans and mice. Thus, we performed experiments to assess effects of $PM_{2.5}$ exposure on the recruitment/mobilization of EPC. Mice (male; C57BL/6; 12-wks) were exposed up to 30 consecutive days (6h/day) to HEPA-filtered air or CAPs (≈8-fold conc.; 80-100 μg/m^3) of urban Louisville air using a versatile aerosol concentration enrichment system (VACES). CAPs exposure for 4, 9 or 30 days significantly decreased the number of circulating EPC measured as Flk-1$^+$/Sca-1$^+$-cells by FACS by 32.6±13.8, 35.8±10.1 or 46±16.9 (% control), respectively. EPC levels returned to baseline within 1 week following a 9day CAPs exposure, indicating a reversible PM effect. CAPs exposure (9-day) significantly increased bone marrow-derived Flk-1$^+$/Sca-1$^+$-cells by 1.75±0.1-fold when compared with controls. Similarly, CAPs exposure (9-day) significantly increased colony forming units (1.6±0.1-fold) and the number of Flk-1$^+$/Sca-1$^+$ or acLDL$^+$/UE-lectin$^+$ cells (1.8 ± 0.1 or 1.6 ± 0.1 fold) of bone marrow-derived cells in culture compared with controls. These results indicated that PM blocked EPC mobilization. To test this, mice exposed for 9 days to air or CAPs were subjected to an EPC mobilization protocol (VEGF, 100μg/kg/d * 4d and AMD3100, 5mg/kg) and blood levels of EPC were analyzed. VEGF/AMD3100 treatment doubled the number of circulating Flk-1$^+$/Sca-1$^+$-cells in air controls but not in CAPs-exposed mice (-48±14 % EPC). These results indicate that inhalation of $PM_{2.5}$ affects EPC mobilization leading to suppressed blood EPC level, which implicates EPC as a potential mechanistic link between $PM_{2.5}$ exposure and CVD.

PS 1171 ALDH1B1 IS A MARKER FOR HUMAN COLON CANCER.

Y. Chen[1], A. Matsumoto[1], D. Orlicky[2], S. Singh[1] and V. Vasiliou[1]. [1]*Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO and* [2]*Pathology, University of Colorado Denver, Aurora, CO.*

Aldehyde dehydrogenases (**ALDHs**) belong to a superfamily of NAD(P)$^+$-dependent enzymes, which catalyze the oxidation of various endogenous and exogenous aldehydes to their corresponding acids. Increased expression and/or activity of ALDHs, particularly ALDH1A1, have been reported to occur in human cancers of various origins. It is proposed that ALDH1A1 may play a critical role in cancer stem cells due to its high affinity for the oxidation of retinal. Nevertheless, the identity of ALDH isozymes in contributing to the enhanced ALDH activity in a specific type of human cancers remains to be elucidated. ALDH1B1 has recently been characterized as a mitochondrial ALDH that actively metabolizes acetaldehyde. In this study, we examined the expression of ALDH1A1 and ALDH1B1 in human tumors of colon, lung, breast and ovary using tissue array (**NIH**) by immunohistochemistry. We observed a much higher percentage of immunopositivity for ALDH1B1 (>60%) in these cancerous tissues than that for ALDH1A1 (<40%); remarkably, 39 out of 40 colonic cancer specimens were positive for ALDH1B1 staining. The immunopositivity of ALDH1A1 or ALDH1B1 was scored by their intensity (scale = 0-3) and extensiveness (% of total cancer cells). Based on this scoring system, the expression levels of ALDH1A1 and ALDH1B1 in these human tumor tissues were in following orders: ALDH1B1 in colon > ALDH1B1 in ovary > ALDH1B1 in lung or breast > ALDH1A1 in lung > ALDH1A1 in colon or ovary > ALDH1A1 in breast. This result demonstrates that ALDH1B1 isozyme is more profoundly expressed in studied human cancer tissues than ALDH1A1, particularly in colon cancer. We further examined the mRNA and protein levels of the two ALDHs in six human colon cancer cell lines. ALDH1A1 was found to be abundant in three cell lines, whereas ALDH1B1 was highly expressed in all six cell lines. In conclusion, our study suggests that ALDH1B1 is preferably upregulated in human colon cancer tissues and cell lines, making it a potential marker for human colon cancer. *Supported in part by NIH grant R21AA017754-01.*

PS 1172 DIFFERENTIAL TOXICITIES OF KINASE INHIBITORS (KI) ON BONE MARROW PROGENITORS FROM DIFFERENT SPECIES.

E. Clarke and G. dos Santos. *ReachBio, Seattle, WA.* Sponsor: R. Steigerwalt.

KIs represent a new class of rationally designed drugs. The success of the initial therapeutic KIs in treating leukemia has spurred the development of new KIs for treating various other cancers and inflammation. However, since myelotoxicity is often associated with administering these agents, and as the use of KIs extends to non-oncology applications, there is a need to develop compounds with lower levels of toxicity than previously associated with this drug class. We previously showed that the IC50 value for 6 KIs using the human colony-forming unit-granulocyte macrophage (CFU-GM) assay correlated with clinical neutropenia and could be used to accurately rank the myelotoxic potential of compounds to other members of the same chemical class. Interestingly, studies have revealed significant differences between human, dog, rat and mouse CFU-GM sensitivities to certain pharmaceuticals. Since multiple animal models are often used in toxicity testing, we

have now compared the IC50 values between human, dog, rat and mouse for Imatinib, Erlotinib, Dasatinib, Sorafenib and Sunitinib. Bone marrow cells from each of the species and KIs were mixed with ColonyGel™ methylcellulose-based media containing appropriate species-specific cytokines and plated in 35 mm dishes (n=3). CFU-GM were enumerated on days 7-14 (species dependent) and the IC50 values determined for each drug for each species. IC50 values determined using dog marrow compared well with human values (e.g.IC50 for Imatinib, Dasatinib and Sorafenib were 2.1, 0.01 and 2.3 μM for dog and 2.5, 0.008 and 3.5 μM for human CFU-GM). In addition, the rank order of the 5 KIs was similar in dog and human assays. In contrast, the IC50 values for the same drugs in rat and mouse assays differed significantly (e.g. IC50 for Imatinib, Dasatinib and Sorafenib were 25, 0.32 and 42 μM for rat and 67, 0.9 and 120 μM for mouse CFU-GM) and the compounds did not rank in the same order. These data suggest that rat and mouse derived IC50 values for KIs may not accurately reflect the relative toxicity of newly designed molecules in this compound class.

PS 1173 DEVELOPMENT AND CHARACTERIZATION OF HUMAN HEPATOCYTES DERIVED FROM INDUCED PLURIPOTENT STEM CELLS (IPSC): A NOVEL *IN VITRO* MODEL SYSTEM FOR ASSESSING DRUG-INDUCED HEPATOTOXICITY.

V. L. Ott, P. Fuhrken, J. Luebke-Wheeler, C. Kannemeier, B. Swanson, W. Wang and E. Nuwaysir. *Cellular Dynamics International, Madison, WI.* Sponsor: K. Kolaja.

Hepatotoxicity and drug-induced liver injury (DILI) are the most common reasons for drug failure during development and market withdrawal of approved drugs. Liver diseases associated with drug toxicity can be attributed, in large part, to the lack of biologically relevant and predictive model systems. Current hepatocyte model systems include primary human hepatocytes (PHH) harvested from cadavers, immortalized cell lines and animal models. Each of these presents limitations in functionality, reproducibility and/or availability. Human hepatocytes derived from induced pluripotent stem cells (iPSCs), which are stem cells derived from adult tissue, offer the potential to overcome these limitations. We have developed human iPSC-derived hepatocytes that are >95% pure and exhibit characteristic hepatocyte morphology, gene and protein expression (e.g. albumin, alpha-1-antitrypsin, ASGR1, HNF family transcription factors). Human iPSC-derived hepatocytes produce albumin protein at levels similar to PHH and ~7-fold greater than HepG2 cells. In addition, >50% of the cells store glycogen and lipid, as demonstrated by Periodic Acid Schiff and Oil Red staining, respectively. Human iPSC-derived hepatocytes express Phase I and II metabolic enzymes (e.g. CYP1A2, 2B6, 2C8/9/19, 2D6, 3A4, UGT, ST and GST) at levels similar to PHH, and CYP3A4 activity is induced ≥3-fold in response to dexamethasone and rifampicin. Finally, human iPSC-derived hepatocytes express key uptake and efflux transporters (e.g. MDR-1/P-gp, MRP2, BSEP, BCRP, NTCP and OATPs) and exhibit formation of bile canaliculi. The development of human iPSC-derived hepatocotyes that recapitulate in vivo hepatocyte function will enable better predictivity of drug-induced hepatotoxicity during the earlier pre-clinical stages of drug development, more successful clinical trials, and the delivery of new drug therapieeis across a wide range of diseases.

PS 1174 BIOLOGICAL SURFACE ADSORPTION INDEX (BSAI) FOR CHARACTERIZING CARBON NANOMATERIALS WITH DIFFERENT SURFACE CHEMISTRIES IN BIOLOGICAL SYSTEMS.

X. Xia, N. A. Monteiro-Riviere and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

The behavior of nanomaterials in a biological or environmental system is governed by the molecular interactions of their surface species with the biological or environmental components. Quantitative assessment of the adsorption properties of nanomaterials is a crucial step for developing predictive structure-activity relationship in nanomedicine and risk assessment of nanomaterials. We have developed a biological surface adsorption index (BSAI) approach to characterize the surface activity of nanomaterials in biological systems. A set of small molecules having diverse physicochemical properties was used as probe compounds. The adsorption coefficients (k) of the probe compounds were obtained by measuring the quantities of the probe compounds adsorbed on the surfaces of the nanomaterials and the equilibrium concentrations of the probe compounds in the media. The log (k) values were scaled to a set of solvation molecular descriptors of the probe compounds via multiple linear regressions to provide a set of five nano-descriptors representing the contributions of the five types of molecular interactions (hydrophobicity, hydrogen-bond acidity and basicity, dipolarity/polarizability, and lone pair electrons). The nano-descriptors for multi-walled carbon nanomaterials (MWCNT) with different surface chemistries (unmodified, -OH and –COOH modified) and fullerenes were measured; for example, the regression model obtained for MWCNT (-OH modified) was $\log(k) = 0.77R + 2.55\pi - 0.14\alpha - 2.36\beta + 4.90V$; n=30, R^2=0.89. The measured nano-descriptors can be used to develop predictive structure-activity relationships in nanomedicine and nanomaterial risk assessments. (Supported by US EPA STAR Grant# R833328 and NIH R01 ES016138)

PS 1175 CELLULAR UPTAKE MECHANISMS AND CYTOTOXICITY OF QUANTUM DOT NANOPARTICLES IN PORCINE DENDRITIC CELLS.

L. W. Zhang and N. A. Monteiro-Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Quantum dots (QD) serve as excellent fluorescent probes and are novel tools used in biomedical applications but the toxicity and mechanism of cellular uptake are poorly understood. Peripheral blood mononuclear cells were isolated from porcine blood by gradient centrifugation and monocytes were purified with anti-CD14 antibody conjugated beads. Monocytes dosed with 0.05nM of QD655 coated with carboxylic acid (QD655-COOH, 18nm) for 30min showed cellular uptake while lymphocytes did not take up QD655-COOH. Monocytes were differentiated into dendritic cells (DC) with granulocyte-macrophage colony stimulating factor and IL-4. Cellular uptake of DC increased six-fold compared to monocytes when cells were dosed with 2nM of QD655-COOH for 30min. QD655 coated with polyethylene glycol (PEG) (45nm) or QD655 PEG-amine (20nm) did not show endocytic uptake in monocytes or DC. QD655-COOH uptake in DC was saturated at 4-6h and TEM showed QD in the cytoplasmic vacuoles of DC. Twelve endocytic inhibitors were used to investigate the cellular uptake mechanisms of QD655-COOH in DC. These results showed that cytochalasin D and chlorpromazine inhibited QD uptake by 50%, demonstrating that cytoskeleton F-actin and clathrin regulated the QD655-COOH endocytosis of DC. The cellular uptake was primarily regulated by scavenger receptor and phospholipase C. Scavenger receptor and a phospholipase c (U-73122) inhibitors blocked QD uptake. In addition, DC maturation with lipopolysaccharide (LPS) caused an increase in QD655-COOH uptake compared to DC without LPS. Viability assays including 96AQ, CCK-8, alamarBlue and ApoTox exhibited minimal toxicity in DC dosed with QD655-COOH at 24h. However, glutathione levels showed a significant decrease with 10nM of QD655-COOH. Lastly, QD655-COOH not only suppressed CD80/CD86 expression on the surface of DC but decreased LPS induced CD80/CD86 expression. These findings shed light on the cytotoxicity and mechanisms of QD cellular uptake in DC.

PS 1176 EVALUATION OF TOXICITY AND INFLAMMATION IN THREE DIFFERENT HYDROXYLATED FULLERENES ($C_{60}(OH)_X$) IN HUMAN CELLS.

J. G. Saathoff[1], X. Xia[1], J. E. Riviere[1], A. O. Inman[1], A. R. Badireddy[2], M. R. Wiesner[2] and N. A. Monteiro-Riviere[1]. [1]*Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC and* [2]*Civil and Environmental Engineering, Duke University, Durham, NC.*

Carbon fullerenes (C_{60}) possess unique chemical properties and their interactions with biomolecules have widespread applications in biomedical technologies. Functionalization of C_{60} with hydroxyl groups can increase the solubility and potential for cellular interaction. There is much speculation as to the potential benefits of the medical application of solubilized fullerenes (i.e. MRI contrast agents, target drug delivery, etc.), and although industrial use and production continues to increase the health and safety effects of hydroxylated C_{60} within biological systems is poorly understood. Conflicting reports regarding the cytotoxicity and inflammation of functionalized C_{60} exists. Hydroxylated fullerenes ($C_{60}(OH)_x$) were synthesized using the prepared phase transfer method. In an attempt to further elucidate the potential for toxicity of functionalized C_{60}, human epidermal keratinocytes (HEK) were exposed to three different hydroxylated fullerenes. $C_{60}(OH)_{20}$, $C_{60}(OH)_{24}$, and $C_{60}(OH)_{32}$ at concentrations ranging from 0.000544 to 42.5μg/ml for 24 and 48hr (n=12 wells/treatment) were exposed to HEK, and alamarBlue (aB) viability assay and IL-8 cytokine analysis were conducted. A statistically significant ($p<0.05$) decrease in viability was seen with $C_{60}(OH)_{24}$ at 8.5μg/ml after 48hr, and $C_{60}(OH)_{32}$ at 42.5μg/ml after 24hr with no effect at 48hr. $C_{60}(OH)_{20}$ had no decrease in viability at any concentration. $C_{60}(OH)_x$ controls

(with and without cells) showed a slight interaction with the aB assay. UV-Vis spectra determined for aB with the three $C_{60}(OH)_x$ showed no effect. Cytokine analysis for all $C_{60}(OH)_x$ showed a slight increase in IL-8 but was not statistically significant. These results suggest that hydroxylation of C_{60} causes minimal cytotoxicity at high hydroxylation levels and no inflammatory response in HEK. (Supported by NIH RO1 ES016138)

PS 1177 THE TRANSCRIPTIONAL PROFILING IN RESPONSE TO C(60) FULLERENES OF RAT LUNGS FOR IDENTIFICATION POTENTIAL BIOMARKERS.

K. Fujita[1], Y. Morimoto[2], S. Endoh[1], K. Uchida[1], H. Fukui[1], A. Ogami[2], I. Tanaka[2], M. Horie[1], Y. Yoshida[1], H. Iwahashi[1] and J. Nakanishi[1]. [1]*National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan and* [2]*University of Occupational and Environmental Health, Kitakyushu, Japan.* Sponsor: M. Takahashi.

In various industrial fields, the use of C(60) fullerenes is expected to increase, however, little is known about the potential toxicological mechanism of action. In our previous study, the transcriptomic profiling data closely matched the pathological findings that C(60) fullerenes caused no serious adverse pulmonary effects after whole-body inhalation exposure. Taking advantage of this, we attempted to characterize time-dependent changes in the gene expression profiles after intratracheal instillation with C(60) fullerenes at different dosages and to identify the candidate expressed genes as potential biomarkers. The hierarchical cluster analysis revealed that the up- or downregulation of genes after intratracheal instillation with 1.0mg C(60) fullerene particles in rat lung tissue was significantly over-represented in the "response to stimulus" and "response to chemical stimulus" categories of biological processes and in the "extracellular space" category of the cellular component. These results were remarkable for 1 week after the instillation with C(60) fullerenes. In the lung tissues instilled with 1.0mg C(60) fullerene particles, many representative genes involved in "inflammatory response" and in "matrix metalloproteinase activity" were upregulated for over 6 months. The expression levels of 89 and 21 genes were positively correlated with the C(60) fullerene dose at 1 week and 6 months after the instillation, respectively. Most of them were involved in "inflammatory response", and the Ccl17, Ctsk, Cxcl2, Cxcl6, Lcn6, Orm1, Rnase9, Slc26a4, Spp1, Mmp7, and Mmp12 genes were overlapped. Microarray-based gene expression profiling revealed that the expression of some genes is correlated with the dose of intratracheally instilled C(60) fullerenes. We suggest that these genes are useful for identifying potential biomarkers in acute-phase or persistent responses to C(60) fullerenes in the lung tissue.

PS 1178 EFFECT OF SURFACE MODIFICATION ON THE BIOAVAILABILITY AND INFLAMMATORY POTENTIAL OF MULTI-WALLED CARBON NANOTUBES.

T. M. Sager[1, 2], M. Wolfarth[1], D. Porter[1], V. Castranova[1], N. Wu[3] and A. Holian[2]. [1]*Pathology/Physiology Reasearch Branch, Health Effects Laboratory Division, NIOSH, Morgantown, WV,* [2]*Center for Environmental Health Sciences, University of Montana School of Medicine, Missoula, MT and* [3]*Department of Mechanical and Aerospace Engineering, West Virginia University, Morgantown, WV.*

Manufactured nanoparticles display physiochemical characteristics and impart unique electrical, thermal, mechanical, and imaging properties that are highly desirable for applications within the commercial, medical and environmental sectors. Carbon nanotubes exhibit excellent electrical, optical and chemical properties with a broad range of applications. In addition, surface modification of carbon nanotubes with organic functional molecules extends their already broad applications. The addition of organic coatings such as –COOH terminated coatings alter the charge, functionality, and reactivity of the nanotube. However, the development of these engineered carbon nanotubes has surpassed evaluation of the potential health impacts. Therefore, this study examines whether carbon nanotubes with different surface chemistry affects pulmonary toxicity. Mice (C57BL/6J, male,7 weeks old) were given 0-40 ug/mouse of either bare mulit-walled carbon nanotubes (MWCNT) or COOH-coated MWCNT via pharyngeal aspiration. Inflammatory and cytotoxic potential of each particle type was analyzed at 1 and 7 days post-exposure. The findings of this study show both the bare and the COOH-modified MWCNTs produced a dose dependent increase in PMNs harvested by whole lung lavage at both 1 and 7 days post-exposure. However, even though the COOH-modified MWCNT did produce a dose dependent increase in PMN number at all doses and at all post-exposure time points, the inflammatory response produced by the COOH-modified MWCNT was lower than the inflammatory response generated by the bare MWCNT. In conclusion, altering the surface functionality of the MWCNT, i.e. bare versus COOH-modified MWCNT lowered the inflammatory properties of the MWCNT. Supported in part by grant RC2 ES-018742 (A. Holian, PI) and NSF grant CBET-0834233 (N. Wu, PI).

PS 1179 OXIDATIVE LIPIDOMICS REVEALS SELECTIVE, BUT NOT RANDOM, PULMONARY PHOSPHILIPID PEROXIDATION AFTER INHALATION OF CARBON NANOTUBES.

Y. Tyurina[1], V. Tyurin[1], L. Sparvero[1], A. Amoscato[1], V. Kapralova[1], E. Kisin[2], A. Murray[2], J. Shi[3], B. Fadeel[3], A. Shvedova[2] and V. Kagan[1]. [1]*Center for Free Radical and Antioxidant Health, Department of EOH, University of Pittsburgh, Pittsburgh, PA,* [2]*Pathology and Physiology Research Branch, Health Effects Laboratory Division, NIOSH, Morgantown, WV and* [3]*Division of Biochemical Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

It is commonly accepted that lipid peroxidation is a mechanism of cell/tissue damage and death by nanoparticles, including single walled carbon nanotubes (SWCNT). We performed global ipidomics/oxidative lipidomics analysis of all molecular species in major classes of phospholipids and established that random free radical peroxidation does not happen in the lung of mice at 3 different time-points after SWCNT inhalation. C57BL/6 mice were exposed to SWCNT via inhalation (5mg/m3, whole body inhalation for 4 consecutive days, 5 h/day) and scarified 1, 7 and 28 days thereafter. We found that two most abundant and susceptible phospholipids - phosphatidylcholine and phosphatidylethanolamine - that constitute more than 75% of total phospholipids, do not undergo peroxidation at all. In addition, we demonstrated that three relatively minor anionic phospholipids - cardiolipin, phosphatidylinositol, and phosphatidylserine get oxidized in a very selective way: only few molecular species out of many in each class (10 out of 25 for CL, 3 out of 5 for PI, 1 out of 7 for PS) were converted into oxygenated species. Moreover, the selectivity was also detected towards fatty acids of the respective oxidized anionic phospholipids (not most polyunsaturated fatty acids but most abundant). We speculate that SWCNT-induced phospholipid peroxidation in lung is involved in the production of selective precursors of pro-resolving mediators of inflammation rather than in non-specific oxidative damage. Supported by NIOSH OH008282; NIH NIAID U19 AI068021, HL70755, HL094488, EC-FP7-NANOMMUNE-214281.

PS 1180 COMPARATIVE GENOTOXICITY OF FIBROUS PARTICLES: CARBON NANOFIBERS, SINGLE-WALLED CARBON NANOTUBES, AND ASBESTOS.

E. Kisin[1], A. R. Murray[1], L. Sargent[2], D. Lowry[2], K. Siegrist[2], M. Chirila[3], D. Schwegler-Berry[1], S. Leonard[1], V. Castranova[1], B. Fadeel[4], V. E. Kagan[5] and A. A. Shvedova[1]. [1]*PPRB, NIOSH, Morgantown, WV,* [2]*TMBB, NIOSH, Morgantown, WV,* [3]*AEB, NIOSH, Morgantown, WV,* [4]*Institute of Environmental Medicine, Karolinska Instituet, Stockholm, Sweden and* [5]*University of Pittsburgh, Pittsburgh, OR.*

Carbon nanofibers (CNF) are generating great interest in industrial sectors such as energy production and electronics, where alternative materials may have limited performance or are produced at a much higher cost. However, despite the increasing industrial use of carbon nanofibers, information on their potential adverse health effects is limited. In the current study, we examine the cytotoxic and genotoxic potential of CNF (Pyrograf®-III) and compare it with the effects of asbestos fibers (crocidolite) or single-walled carbon nanotubes (SWCNT). The genotoxic effects in hamster lung fibroblast (V79) cell line were examined using two complementary assays: comet and micronucleus (MN). In addition, we utilized fluorescence *in situ* hybridization to detect the chromatin pan-centromeric signals within the MN. The use of this probe allows the classification of MN as being centromere positive or centromere negative indicating their origin by anaugenic (chromosomal malsegregation) or clastogenic (chromosome breakage) mechanisms, respectively. Results from cytotoxicity tests revealed a concentration- and time-dependent loss of cell viability after exposure to all tested materials in the following sequence: asbestos>CNF>SWCNT. Additionally, cellular uptake and generation of oxygen radicals was seen in the murine RAW264.7 macrophage cell line following exposure to CNF or asbestos but not after administration of SWCNT. Comet and MN assays demonstrated the induction of DNA damage and MN after exposure to all tested materials with the strongest effect seen for CNF. Moreover, the chromatin pan-centromeric signals within the MN were positive after cell exposure to CNF suggesting that the mitotic spindle and the segregation of the chromosomes were affected. Supported by NIOSH OH008282, NORA 0HELD015, and EC-FP-7-NANOMMUNE-214281.

PS 1181 ELUCIDATION OF FACTORS DETERMINING CARBON NANOTUBES' ABILITY TO PENETRATE ALVEOLAR EPITHELIAL BARRIER AND INTERACT WITH LUNG FIBROBLASTS *IN VITRO.*

R. Derk[1], A. Mishra[1], T. Stueckle[1], Y. Rojanasakul[2], V. Castranova[1] and L. Wang[1]. [1]*HELD/PPRB, CDC/NIOSH, Morgantown, WV and* [2]*School of Pharmacy, West Virginia University, Morgantown, WV.*

Nanomaterials possess unique physicochemical and biological properties but can also exhibit different adverse reactions if inhaled. Our previous *in vivo* study showed upon alveolar deposition, dispersed single-walled carbon nanotubes (DSWCNT) rapidly enter interstitial area (1 day post-exposure) and induce interstitial fibrotic response as early as 1 week post-exposure. Direct stimulation of cultured lung fibroblasts, a major interstitial cell, by DSWCNT was shown to enhance proliferation and collagen production, a hallmark of lung fibrosis. Furthermore, penetration of DSWCNT through lung epithelial barrier into interstitium could be a key event of DSWCNT-induced interstitial fibrosis. To investigate this alveolar epithelial barrier, an experimental model was developed using immortalized human lung epithelial cell line (ATCC, Manassas, VA). Epithelial cells were cultured on the apical surface of Transwell® microporous membrane and exposed to non-dispersed SWCNT and DSWCNT. Samples from the apical compartment, cell monolayer, and basolateral compartment were collected at various times and analyzed for CNT penetrability. Electron microscopy and CytoViva hyperspectral imaging were used to aid characterization of the penetration pathway (paracellular vs. transcellular) of nanoparticles across alveolar epithelial membrane. The effect of CNT dispersion status on penetration rate was also assessed. Our data suggest CNT penetrated through epithelial cells on apical side and translocated to the other side of the Transwell membrane and the amount of CNT transferred, measured by hyperspectral imaging, was sufficient to induce fibroblast proliferation and collagen production based on previous data. The Transwell system is a suitable model for studying translocation of CNT across epithelial layer and aids in mechanistic studies of CNT- induced interstitial lung fibrosis.

PS 1182 *IN VITRO* ASSESSMENT OF POTENTIAL TUMORGENICITY OF CHRONIC SWCNT AND MWCNT EXPOSURE TO LUNG EPITHELIUM.

T. A. Stueckle[1], A. Mishra[1], R. Derk[1], Y. Rojanasakul[2], V. Castranova[1] and L. Wang[1]. [1]*HELD/PPRB, CDC/NIOSH, Morgantown, WV and* [2]*School of Pharmacy, West Virginia University, Morgantown, WV.*

Carbon nanotube use in multiple consumer and industrial products has resulted in increased concern for long-term risks to human health. Recent *in vivo* studies suggest that inhaled carbon nanotubes induce interstitial fibrosis and persist in exposed lung tissues. No clear evidence or experimental model exists to assess whether long-term pulmonary exposures of CNT to lung tissue results in transformed cells expressing a tumorigenic phenotype. This study tested the hypothesis that chronic exposure to dispersed carbon nanotubes induces neoplastic transformation in lung epithelial cells *in vitro.* Small airway lung epithelial cells were exposed for 25 weeks to either dispersed single wall (D-SWCNT) or multi-wall (D-MWCNT) carbon nanotubes at non-cytotoxic levels (0.02 $\mu g/cm^2$). Dispersed ultra fine carbon black (D-CB) and asbestsos (ABS) served as negative and positive controls. Following exposure each treatment was evaluated for tumorigenic phenotypes using cell proliferation, cell invasion and colony formation assays in untreated media. D-SWCNT exhibited a significant 1.5-fold increase in cell proliferation compared to passage control cells while D-MWCNT and D-CB showed a modest increase. In addition, D-SWCNT cells displayed significantly greater invasive potential than D-MWCNT, ABS and passage control. Lastly, a tumor formation assay resulted in D-SWCNT cells possessing a significant 5-fold increase in the number of colonies formed above controls while D-MWCNT and D-CB exhibited only a 1.5-fold increase. Our chronic, low dose *in vitro* exposure model suggested that D-SWCNT exposure caused neoplastic transformation resulting in a tumorigenic phenotype while D-MWCNT exposure displayed less malignant potential. In conclusion, use of *in vitro* screening methods, along with comparable *in vivo* data, can assist in investigation of tumorigenic risks associated with carbon nanotube and other nanomaterial exposures.

PS 1183 ASSESSMENT OF FIBROGENIC BIOMARKERS INDUCED BY MULTI WALL CARBON NANOTUBES.

A. Mishra[1, 2], Y. Rojanasakul[2], V. Castranova[1, 2], R. Mercer[1] and L. Wang[1, 2]. [1]*HELD/PPRB, CDC/NIOSH, Morgantown, WV and* [2]*Pharmaceuticals & Pharmacological Sciences, West Virginia University, Morgantown, WV.*

Multi Wall Carbon Nanotubes (MWCNT), a graphene based nanoparticle, possess unique physiochemical properties. Considered as a technological breakthrough, production of MWCNT is rapidly increasing worldwide but toxicity profile of the nanomaterials is not clearly understood. Among the adverse effects, CNT have been shown to induce (the development of unusual) interstitial lung fibrosis at physiologically relevant exposure (10μg/mouse); however, the underlying mechanism is not fully known. In this present study, we investigated important MWCNT-induced fibrogenic mediators using cultured lung cell systems. Human bronchial epithelial (BEAS-2B) cells, alveolar epithelial (A549) cells, and lung fibroblast (CRL1490) cells were treated with MWCNT. Viability of MWCNT-exposed cells was determined by cell counting and WST-1 assay. Fibrogenic mediators, including Fibroblast Growth Factor-2, Vascular Growth Factor, Transforming Growth Factor β1 (TGF-β1), and Platelet Derived Growth Factor-A, were analyzed using Western Blots and end point ELISA. Our results show that 1) MWCNT decreased cell viability of epithelial cells in a dose and time dependent manner, 2) MWCNT exposure induced secretion of fibrogenic mediators from lung epithelial cells and fibroblasts at physiologically relevant concentrations of 0.02 to 0.6 $\mu g/cm^2$ and 3) MWCNT directly induced collagen production from lung fibroblasts. In conclusion, MWCNT induced fibrogenic mediators in cultured human lung epithelial cells and stimulated collagen production from fibroblasts. These data are consistent with *in vivo* observations. Therefore, the *in-vitro* cell culture systems can be used for mechanistic studies and screening tests for MWCNT and similar fibrogenic nanoparticles.

PS 1184 DIFFERENTIAL EFFECTS OF SINGLE-WALLED CARBON NANOTUBES ON HUMAN HEPATIC, RENAL, AND COLORECTAL CARCINOMA CELL LINES.

K. Hitoshi[1], M. Katoh[1], T. Suzuki[2], Y. Ando[1] and M. Nadai[2]. [1]*Faculty of Pharmacy, Meijo University, Nagoya, Japan and* [2]*Faculty of Science and Technology, Meijo University, Nagoya, Japan.*

Carbon nanotubes (CNTs) are currently one of the promising materials for the development of nano-technologies. However, CNT toxicity is a major concern. Many in vitro studies have assessed the cytotoxicity of CNTs, but the results differ according to the cell lines and catalysts used for synthesizing CNTs. We have previously reported the differential effects of single-walled CNTs (SWCNTs) on human lung carcinoma A549 and human head and neck carcinoma FaDu cells (SOT 2010 annual meeting). The present study aimed to clarify the cytotoxic effects of SWCNTs on HepG2, ACHN, and Caco-2 cells derived from the human liver, kidney, and colon, respectively, because SWCNTs are expected to be used as drug and gene carriers in medical fields. The SWCNTs used in this study were manufactured using 2 types of arc electrical discharge method, with Ni and Y (SO-SWCNTs) and Fe (FH-P-SWCNTs) as catalysts. Cell viability was evaluated on the basis of the ATP content and metabolic capacity of the cell. SWCNTs were exposed to the cells at concentrations of up to 1.0 mg/ml. On 24-h exposure of 1.0 mg/ml SO-SWCNTs to HepG2, ACHN, and Caco-2 cells, the ATP content of these cells decreased to 91%, 87%, and 90% of the ATP content of untreated cells, respectively. Under identical conditions, HepG2, ACHN, and Caco-2 cells exposed to FH-P-SWCNTs showed similar results (decrease in ATP content to 91%, 97%, and 93%, respectively). However, the metabolic capacity of SO-SWCNT-exposed cells was slightly higher than that of FH-P-SWCNT-exposed cells, that is, 63%, 88%, and 69% vs. 50%, 93%, and 48% for HepG2, ACHN, and Caco-2 cells, respectively. These results suggest that exposure to SWCNTs has a greater effect on metabolic activity than on ATP content of these cells and that ACHN cells are most resistant to SWCNT exposure. The present study clarified that the effects of SWCNTs on cell viability differed depending on the type of cell, SWCNT, and analytical method used to assess cell viability.

PS 1185 MULTI-WALL CARBON NANOTUBE (MWCNT)-INDUCED GENE EXPRESSION IN THE MOUSE LUNG: IMPLICATION OF CARCINOGENESIS RISK.

M. Pacurari[2, 1], Y. Qian[1], A. Hubbs[1], D. Porter[1], M. Wolfarth[1], D. Luo[2], Y. Wan[2], V. Castranova[1] and N. Guo[2, 3]. [1]*Pathology and Physiology Research Branch, NIOSH, Morgantown, WV,* [2]*MBR Cancer Center, West Virginia University, Morgantown, WV and* [3]*Department of Community Medicine, West Virginia University, Morgantown, WV.*

MWCNT's fibrous-like shape and durability suggest they may pose a similar carcinogenic effect on humans as asbestos. Therefore, this study sought to investigate how previously identified lung cancer prognostic biomarkers and the related cancer signaling pathways are affected in the mouse lung following in vivo pharyngeal aspiration of MWCNT. A total of 63 identified lung cancer prognostic biomarker genes were analyzed using quantitative PCR assays in the mouse lung exposed by aspiration to 0, 10, 20, 40, 80 μg of MWCNT at 7-days and 56-days post-exposure. At 7- and 56-days post-exposures, a set of 7 genes and a set of 11 genes showed significant differential expression in the mouse lungs exposed to MWCNT vs. unexposed control groups, respectively. These significant genes could clearly separate

the control group from the treated group over the time series in hierarchical gene clustering analysis. Furthermore, 4 genes from these two sets of significant genes, Ccdc99, Msx2, Nos2 and Wif1, showed significant mRNA expression perturbations at both time points. It was also found that the expression changes of these 4 overlapped genes at 7-days post-exposure were attenuated at 56-days post-exposure. The results of MWCNT exposure-induced gene expression changes reveal the characteristics of carcinogenesis and may indicate the association of MWCNT exposure with lung cancer progression. These results also indicate that MWCNT exposure may induce the alteration of several key carcinogenesis-related signaling transduction pathways. Taken together, the results obtained from this study indicate the potential lung carcinogenetic effects of MWCNT exposure in humans and could potentially be used for the medical surveillance for MWCNT workers.

PS 1186 MULTI-WALLED CARBON NANOTUBE INSTILLATION IN C57BL/6 MICE INDUCES CHANGES IN PULMONARY FUNCTION.

P. Katwa[1], A. Aldossari[1], S. C. Hilderbrand[1], C. J. Wingard[2], D. M. Walters[2] and J. M. Brown[1]. *[1]Pharmacology & Toxicology, East Carolina University, Greenville, NC and [2]Physiology, East Carolina University, Greenville, NC.*

As the production and use of nanomaterials increases, the potential for human exposure also increases thereby placing greater emphasis on potential health hazards. Multi-walled carbon nanotubes (MWCNT) in particular, are widely used for their versatility in many disciplines due to their unique physical and chemical properties. The purpose of this study was to examine the long-term pulmonary inflammatory and fibrotic effects of MWCNT at 7, 30, 60 and 90 days post-exposure. Inflammatory and fibrotic responses in lungs of C57BL/6 mice following MWCNT instillation were assessed by cytokine expression, bronchoalveolar lavage cell counts, collagen content, pulmonary function testing and histological assessment. Mice instilled with MWCNT (100 μg) exhibited increased expression of pro-inflammatory and pro-fibrotic cytokines, which were associated with increased infiltration of macrophages, neutrophils, eosinophils and lymphocytes up to 60 days post-exposure. In addition, pulmonary function testing demonstrated an increase in the area within the pressure-volume loops (PV loops) for MWCNT treated mice compared to vehicle control indicating a change in lung hysteresis. These findings corroborate histological data exhibiting increased granuloma formation and development of fibrosis up to 90 days associated with increased collagen content in lung tissue of C57BL/6 mice instilled with MWCNT. These data indicate that exposure to MWCNT results in adverse pulmonary effects, including increased pulmonary inflammation, fibrosis and compromised lung function. This work supported by NIH RO1 ES019311 (JMB) and ES016246 (CJW).

PS 1187 CARBON NANOTUBES INDUCE APOPTOSIS RESISTANCE THROUGH FLICE-INHIBITORY PROTEIN.

V. Pongrakhananon[1], Y. Lu[1], L. Wang[2], T. Stueckle[2], S. Luanpitpong[1] and Y. Rojanasakul[1]. *[1]West Virginia University, Morgantown, WV and [2]National Institute for Occupational Safety and Health, Morgantown, WV.*

Our studies have shown that chronic exposure to single-walled carbon nanotubes (SWCNT) induces apoptosis resistance and malignant transformation of human lung epithelial cells. Since resistance to apoptosis is a foundation of neoplastic evolution and selection of malignant transformed phenotype, we investigated the apoptosis pathway underlying the resistance its mechanisms to aid the understanding of SWCNT-induced carcinogenesis. As compared to passage-matched control cells, SWCNT-transformed BEAS-2B cells exhibited resistance to apoptosis induced by death ligands, e.g., tumor necrosis factor-a and Fas ligand, but not by inducers of mitochondria-mediated apoptosis, e.g., antimycin A and cisplatin, suggesting death receptor pathway as the primary pathway of defective apoptosis in SWCNT-transformed cells. The results were confirmed using caspase specific inhibitors and caspase activity assays. DNA microarray and Western blot analyses of key apoptosis-regulatory proteins in the transformed cells revealed FLICE-inhibitory protein (FLIP) as an important target of regulation by SWCNT. Overexpression of FLIP increased apoptosis resistance of the cells, whereas RNAi knockdown of FLIP reversed the apoptosis resistance of cells in response to death ligands. Together, our study demonstrated a novel mechanism of apoptosis resistance induced by chronic exposure to SWCNT in human lung epithelial cells and identified FLIP as a key regulator of apoptosis avoidance that contributes to the development of malignant transformed phenotype. [This work is supported by the NIH grant R01-HL095579]

PS 1188 POTENTIAL CARCINOGENICITY OF CARBON NANOTUBES.

Y. Rojanasakul[2], Y. Lu[2], S. Luanpitpong[2], V. Castranova[1], V. Pongrakhananon[2] and L. Wang[1]. *[1]National Institute for Occupational Safety and Health, Morgantown, WV and [2]West Virginia University, Morgantown, WV.*

Carbon nanotubes (CNT) have increasing been used for wide applications with a potential for human exposure. Concerns about the potential carcinogenicity of CNT have been raised since CNT exhibit a bio-persistence and a fibrous morphology similar to asbestos which is a known carcinogen. However, there is neither clear knowledge nor a practical method to assess this potential. In this study, we developed an in vitro chronic exposure model combined with in vivo xenograft model to address these needs. Non-tumorigenic human lung epithelial BEAS-2B cells were continuously exposed to a sub-cytotoxic concentration (0.04 ug/ml or 0.02 ug/cm2 exposed area) of single-walled CNT (SWCNT) in culture. Phenotypic changes were observed in SWCNT-treated cells 20 weeks post-exposure such as formation of cell mounds and accelerated cell growth. SWCNT-treated cells were subsequently analyzed for malignant properties including colony formation, cell migration and invasive properties. Significant positive results were observed from SWCNT-treated cells in all above studies compared to passage-matched control cells. In vivo tumorigenesis study was performed by subcutaneously injecting the transformed cells into nude mice. Consistent with the in vitro cell transformation results, the in vivo results showed large tumor formation at the injection site in mice receiving SWCNT-transformed cells, whereas mice receiving control cells showed no tumor formation. These studies indicate that long-term/low dose exposure of human lung epithelial cells to SWCNT induced malignant transformation of the cells which induced tumor formation in vivo. These results suggest a potential carcinogenic effect of SWCNT. The described cell model system could potentially be used as a predictive model for carcinogenicity testing of nanomaterials. [This work was supported by the NIH Grant R01-HL076340]

PS 1189 PULMONARY EXPOSURE TO CARBONACEOUS NANOPARTICLES AFFECTS LOCAL AND SYSTEMIC IMMUNITY.

A. Tkach[1], E. Kisin[1], A. R. Murray[1], G. V. Shurin[2], M. R. Shurin[2], S. H. Young[1], A. Star[2], B. Fadeel[3], V. E. Kagan[2] and A. A. Shvedova[1]. *[1]PPRB, NIOSH, Morgantown, WV, [2]University of Pittsburgh, Pittsburgh, PA and [3]Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden.*

Numerous studies have focused on the toxicity associated with nanoparticle (NP) exposure. The results of studies on rodents have demonstrated that NP are capable of inducing inflammation, granulomas, fibrosis, and mutagenicity found in the lungs. However, immunologic effects of inhaled nanoparticles remain largely unexplored. In the current study, we evaluated the inflammatory response in the lung and systemic immune effects induced by pulmonary exposure to 40-120 μg/mouse of pristine (C_{60}) or functionalized (C_{60}-TRIS) fullerenes or single-walled carbon nanotubes (SWCNT). We demonstrated that pharyngeal aspiration of the studied NP caused inflammation and pulmonary damage as evidenced by accumulation of PMNs, changes in lung permeability and cell damage. In addition, NP stimulated release of pro-inflammatory and regulatory cytokines in the lung. Further, local inflammatory response was translated into suppressed systemic immunity as evidenced by 25% decrease in proliferation of splenic T cells stimulated by allogeneic dendritic cells (DC). To investigate possible mechanisms of compromised systemic immunity, we evaluated the ability of NP to directly affect stimulatory/polarizing activities of conventional DC (cDC) towards T cells *in vitro*. Exposure of cultured cDC to NP resulted in an impaired ability to stimulate T cells in an allogeneic MLR assay (up to 4-fold suppression). This effect was due to neither altered expression of CD80, CD86 or MHCII on DC as exhibited by DC phenotyping, nor by increased production of IL-10. Thus, mechanisms of altered systemic immunity in treated mice might be, at least in part, due to the direct effects of NP on cDC. Overall, our data suggest that NP affect and trigger both local and systemic immune responses in mice.
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PS 1190 PULMONARY RESPONSE OF CIGARETTE SMOKE-EXPOSED MICE TO CARBON NANOPARTICLES.

C. Gairola[1], S. Han[1] and D. Bhalla[2]. *[1]Graduate Center for Toxicology, University of Kentucky, Lexington, KY and [2]Wayne State University, Detroit, MI.*

Concurrent or sequential exposures to more than one air pollutant can potentially affect pulmonary toxicity. Carbon nanoparticles when instilled into the lungs of mice induce an acute pulmonary response. To determine if such a response is influ-

enced by pre-exposure to cigarette smoke (CS), we exposed female A/J mice to side-stream cigarette smoke for 6hrs/d, 5d/wk for 4 wks and then challenged them with a single dose of 40μg multiwall carbon nanotubes (CNT) in 50 μl of phosphate buffered saline (PBS) by pharyngeal aspiration. Control animals exposed to ambient air received 50 μl PBS. Pulmonary response was compared in a total of four groups: 1) Air/PBS-control, 2) CS/PBS, 3) Air/CNT, and 4) CS/CNT. Bronchoalveolar lavage fluid (BALF) samples were collected at 24 and 72 hrs post-CNT treatment. BALF was analyzed for various markers of cytotoxicity and inflammation using standard ELISA and immunoblot assays. The results showed a pronounced cellular response and increase in various cytotoxicity/inflammatory markers (total BALF protein, LDH, cytokines and mucin) in the lungs of CNT-exposed mice. Pre-exposure to CS for 4wks, by itself, produced minimal effects and generally did not affect cytotoxic responses such as BALF protein and LDH. However, BALF cytokine response to CNT was suppressed in CS-pre-exposed animals in comparison to corresponding air-exposed CNT controls. While cytokine levels returned to baseline by 72hrs,significantly higher levels of BALF mucin persisted in air and CS-exposed mice at 24 and 72 hrs post-CNT administration. These results suggest the development of "cross tolerance" similar to that observed in our earlier studies of ozone-CNT interaction and demonstrate that prior exposures can modulate toxic response (supported in part by funds from OVPR,WSU).

PS 1191 INHALATION OF SINGLE-WALL CARBON NANOTUBES INCREASES APOPTOSIS AND ENDOTHELIN-1 LEVELS IN HAMSTER LUNGS.

K. H. Bijlani, H. R. Sukhija, J. O. Cantor and J. M. Cerreta. *PHS, St. Johns University, Queens, NY.* Sponsor: L. Trombetta.

Single-wall carbon nanotubes (SWCNT) consist of a graphene sheet rolled into a cylinder with diameters of few nanometers (nm) and length that may range up to a micrometer. SWCNT have potential applications as superconductor material and in biosensors, with human exposure occurring in the workplace. The current study was undertaken to evaluate the effects of inhalation exposure of aerosolized SWCNT on hamster lungs. Golden Syrian Hamsters were divided in to 2 groups: a control group that was exposed to an aerosol of autoclaved distilled water, and a treated group that was exposed to aerosolized SWCNT at doses of 1mg/m3, 2mg/m3 and 4mg/m3 for 2hrs/day for 4 consecutive days. Additional groups were used to investigate longer inhalation periods (8 days and 14 days) with exposures of 4hrs/day to 2mg/m3 SWCNT. One day following completion of exposures, hamsters were euthanized and the lungs were either lavaged or removed for histological examination. Bronchoalveolar lavage fluid (BALF) was analyzed for total cell count, and lung tissue sections were stained and examined microscopically. Apoptosis levels in lung tissue sections were determined by TUNEL Assay and endothelin-1 (ET-1) levels were also measured. Compared to control groups, hamsters exposed to inhalation of 1, 2 and 4mg/m3 SWCNT demonstrated an increase in total cell count of 207%, 273% and 279% respectively. Animals treated with 2 and 4mg/m3 also had elevated lung ET-1 levels (179% and 213%) respectively. Hamsters treated for 8 or 14 days had BALF cell counts that were increased to 268 % and 410%, respectively, when compared to controls. Lung ET-1 levels were also increased in the lungs of hamsters treated for 8 days (207%) and 14 days (237%) in comparison to controls. Histological examination revealed an increased number of TUNEL positive cells, alveolar septal thickening and the presence of small granulomatous-like lesions with higher concentrations of SWCNT. Results from this study suggest that inhalation of SWCNT induces pulmonary injury in hamsters.

PS 1192 INHALED SINGLE WALL CARBON NANOTUBES INCREASE LEVELS OF 5–LIPOXYGENASE AND LTB4 IN HAMSTER LUNGS.

H. R. Sukhija, K. H. Bijlani, J. O. Cantor and J. M. Cerreta. *PHS, St. Johns University, Queens, NY.* Sponsor: L. Trombetta.

Single-walled carbon nanotubes (SWCNT) are elongated, cylindrical particles, with a diameter of 1-2nm, that have applications in electronics and biotechnology industries. As such materials become more common in the work place, assessment of their potential hazard becomes important. The current investigation was initiated to study the possible risk posed by inhaled SWCNT to the lung. Separate groups of Golden Syrian Hamsters (6-7 weeks old) were exposed to 1mg/m3, 2mg/m3, and 4mg/m3 of SWCNT for 2hrs/day for 4 days. Further studies exposed animals for 4hrs/day for 8 days and for 14 days with 2mg/m3 SWCNT. All SWCNT-treated groups were compared to their respective vehicle-treated controls at 24 hrs following the exposure period. Hamsters were euthanized and their lungs collected for paraffin-embedding or for lavage. Bronchoalveolar lavage fluids (BALF) were assessed for total cell numbers, 5–lipoxygenase (LOX), and leukotriene B4 (LTB4). Lung tissue sections were evaluated for LOX activity by immunohistochemistry. Hamsters exposed to 1mg/m3, 2mg/m3, and 4mg/m3 of SWCNT had increased: total BALF cell counts of 2, 2.7 and 2.7 fold, respectively, and increased LTB4 levels of 2.9, 5.4 and 3 fold compared to the controls. Animals exposed for 8 days had increased total cell counts (2.6x) and LTB4 levels (11.3x) as compared to the controls. Fourteen-day exposed animals had an increased total cell count (4x) and LTB4 levels (3.7x) as compared to the controls. LOX levels increased in the alveolar septa and in alveolar macrophages as compared to the controls. Differential cell counts demonstrated foamy macrophages and the presence of lymphocytes in the treatment groups that were absent from control groups. Such data indicate that inhaled SWCNT induces pulmonary inflammation in hamsters with activation of LOX and increased levels of LTB4.

PS 1193 AMPHIPHILIC POLYMER-COATED CdSe /ZnS QUANTUM DOTS INDUCE LUNG INFLAMMATION IN MICE WHICH IS MODULATED BY GLUTATHIONE STATUS.

D. Botta, C. C. White, C. S. Weldy, L. A. McConnachie, J. Wilkerson, S. E. Gill, X. Hu, W. C. Parks, X. Gao and T. J. Kavanagh. *University of Washington, Seattle, WA.*

Quantum dots (QDs) are unique semi-conductor fluorescent nanoparticles with potential uses in a variety of biomedical applications. However, concerns have been expressed regarding their potential toxicity, specifically their capacity to induce oxidative stress. In this study we synthesized CdSe/ZnS core/shell QDs with a tri-n-octylphosphine oxide, poly(maleic anhydride-alt-1-tetradecene) (TOPO-PMAT) coating and assessed their effects on the respiratory tract of mice. Previous in vitro data in macrophages had shown these QDs cause mild oxidative stress and secretion of pro-inflammatory cytokines, and that this was glutathione (GSH)-dependent. We therefore investigated their effects in vivo in mice genetically engineered to have deficiencies in GSH (GCLM null mice). When exposed via nasal instillation to a 6 μg/kg Cd equivalent dose of QDs at 8 hours, neutrophil counts in broncho-alveolar lavage fluid (BALF) increased in both wild-type (WT) as well as GCLM heterozygous (HT) mice, whereas GCLM null (KO) mice exhibited no increase in neutrophils. HT mice had a significantly higher level of neutrophils than WT mice. The NOAEL for HT mice was 0.25 μg/kg Cd equivalents. TOPO-PMAT gold nanoparticles had no effect on neutrophil influx in either WT or HT mice. Lung cadmium (Cd) levels peaked at 1 hr in HT mice, but were similar in WT mice at 0.5hr, 1hr and 3hr. Cd levels in KO mice peaked at 0.5 hr. Levels of the pro-inflammatory cytokines KC and TNFα in BALF increased in the WT and HT mice, but not in KO mice. There was no change in matrix mettaloproteinase (MMP) activity in the lungs for any genotype. Neither WT nor HT mice had increased levels of myeloperoxidase (MPO – a marker of neutrophil influx). Interestingly, there was a decrease in MPO in the KO mice (irrespective of treatment) relative to untreated WT mice. We conclude that TOPO-PMAT QDs are pro-inflammatory in vivo in mice in a GSH-dependent manner. Supported by NIH Grants R01ES016189, P30ES07033 and T32ES07032.

PS 1194 BIO-DISTRIBUTION OF FULLERENE INTRAVENOUS ADMINISTRATED IN RAT.

T. Nishimura[1], R. Kubota[1], M. Tahara[1], K. Shimizu[1], T. Obama[1], N. Sugimoto[1] and A. Hirose[2]. [1]*Division of Environmental Chemistry, National Institute of Health Sciences, Tokyo, Japan and* [2]*Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan.*

Engineered nanomaterials are applied to pharmaceuticals, foods, cosmetics, and so on. Among these engineered nanoparticles, it have been increasing the concern about the exposure risk of fullerenes and their derivatives increases in the occupational and/or living environment through the oral, dermal and inhalation route by the rapid commercialization. However, the effect on the human health and the biological behavior has not studied sufficiently. In this study, we have examined the distribution of fullerene after injection into the rat tail vein to mimic the digestive absorption. The fullerene was extracted with toluene from tissues and then measured by liquid chromatography with tandem mass spectrometry. The fullerene was detected in liver, kidney, spleen and lung in all injected rats on both the first day and the fourth day after injection. However, the fullerene was not detected in blood and brain. The highest concentration was detected in the lung on the first day after the injection. The average concentration in lung was almost similar to that in liver and spleen, but much lower in kidney. The concentrations were decreased with the time dependency in all tissues except spleen on the fourth, fourteenth and twenty-eighth days in comparison with on the first day. The concentration in kidney, however, had rapidly decreased on fourth day. In the case of pregnancy rats, the fullerene was also distributed in lung, liver, spleen and kidney. However, the fullerene was also very low in kidney and could not be detected in placenta and fetus. These results indicate that the fullerene absorbed in the body via any route of exposure may distribute to lung, liver, spleen and kidney as the primary exposure

target organs, and the fullerene existed in the blood was possibly excreted rapidly into the urine through kidney. But there is possibility fullerene might be metabolized to metabolites by metabolic enzymes.

PS 1195 AIRWAY INFLAMMATION IN MICE EXPOSED TO OVALBUMIN ALLERGEN AND MULTI-WALLED CARBON NANOTUBES (MWCNT) IS REGULATED BY CYCLOOXYGENASE-2 (COX-2).

B. C. Sayers[1], E. E. Glista[1], A. J. Taylor[1], R. Langenbach[2] and J. C. Bonner[1]. *[1]Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC and [2]Laboratory of Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC.*

Growing evidence suggests inhalation exposure to multi-walled carbon nanotubes (MWCNT) pose a significant human health risk by causing lung injury, inflammation, and fibrosis. We previously showed that inhaled MWCNT cause airway fibrosis in mice that were pre-exposed to ovalbumin (OVA) allergen challenge. Cyclooxygenase-2 (COX-2) is an important mediator of pulmonary fibrosis and asthma. This study was designed to test the hypothesis that COX-2 plays a protective role in airway inflammation and fibrosis in mice exposed to OVA and MWCNT. COX-2-deficient (COX-2-/-) mice or wild-type (COX-2+/+) mice were pre-exposed to saline or OVA prior to oropharyngeal aspiration (OPA) of MWCNT (4 mg/kg) or vehicle (0.1% pluronic in saline). Lungs were collected at 1 and 14 days after MWCNT exposure for histopathology, RNA, protein, and collection of bronchoalveolar lavage fluid (BALF). MWCNT delivered by the OPA method were evenly distributed throughout the lung and contained mainly within alveolar macrophages. Differential counts of BALF cells at 1 day showed that OVA alone, but not MWCNT alone, caused a significant increase in inflammatory cells from either COX-2+/+ or COX-2-/- mice. Combined OVA/MWCNT exposure increased total cell counts in COX-2+/+ above that seen with OVA alone. BALF cell counts were elevated even more in COX-2-/- mice exposed to OVA/MWCNT as compared to COX-2+/+ mice. OVA alone increased IL-13 protein in BALF to a similar level in either COX-2+/+ and COX-2-/- mice (~25 pg/ml) whereas IL-13 was not detectable in saline or MWCNT groups. Combined OVA/MWCNT also increased IL-13 to ~25 pg/ml in COX-2+/+ mice, yet OVA/MWCNT caused a significant increase in IL-13 in COX-2-/- mice (~75 pg/ml). These findings suggest that COX-2 plays an important role in reducing MWCNT-induced airway inflammation in individuals with pre-existing asthma.
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PS 1196 RESPIRATORY EFFECTS OF INHALED SINGLE-WALLED CARBON NANOTUBES: THE ROLE OF IRON AND PARTICLE MORPHOLOGY.

A. K. Madl, A. Tsumura, K. Johnson, V. Seshachellam, P. C. Edwards, S. V. Teague, T. Guo, L. S. Van Winkle, J. E. Evans and K. E. Pinkerton. *University of California, Davis, Davis, CA.*

With worldwide production of carbon nanotubes (CNTs) exceeding over 500 metric tons annually and industry growth expecting to double over the next 5 yr, there are concerns our understanding of the hazards of these nanomaterials may not be keeping pace with market demand. The physicochemical properties of CNTs may delineate the key features that determine either toxicity or biocompatibility and assist in evaluating the potential health risks posed in industrial and consumer product settings. We hypothesized that the iron content and morphology of inhaled single-walled carbon nanotubes (SWCNTs) influences the extent of cellular injury and alters homeostasis in the lung. Rats (SD) were exposed (1 mg/m3) to either aerosolized SWCNTs (raw FeSWCNT or purified cSWCNT), carbon black (CB), crocidolite, or fresh air via nose-only inhalation for 6 hr/d for 10 d. Markers of inflammation and cytotoxicity in lung lavage and mucin in different airway generations were used to assess immediate and persistent effects. The oxidant and inflammatory capacity of microdissected airways of exposed animals was used to assess the ability to withstand an additional oxidant insult. Results showed different timing and extent of responses resulting from exposure to SWCNTs containing varied amounts of iron. Depending on the endpoint of interest, responses of SWCNTs sometimes followed that of CB while in other circumstances matched that of crocidolite. Notably, FeSWCNTs exposed animals were unable to respond to an additional oxidant challenge and cSWCNTs exposed animals had a latent and persistent development of mucous cells in the distal airways. In conclusion, while some toxicity endpoints follow patterns comparable to CB or crocidolite, the respiratory effects of inhaled FeSWCNTs and cSWCNTs appear to be unique. Further research is needed to evaluate whether these changes are suggestive of precursor events to pathologic changes that might develop under more severe or prolonged exposure conditions. UC TSRT&P and RC1ES018232

PS 1197 *IN VIVO* TOXICITY STUDY OF QUANTUM DOT NANOPARTICLES FOLLOWING INTRAVENOUS ADMINISTRATION IN MICE.

J. Treadway[2], J. Bartel[2], J. Schatz[3] and S. Kim[1]. *[1]Design-for-Environment, Life Technologies, Foster City, CA, [2]Life Technologies, Eugene, OR and [3]Design-for-Environment, Life Technologies, Madison, WI.*

Quantum dots provide the revolutionary fluorescence performance inherent in the nanocrystal structure with a highly customizable surface for directing the bioactivity of the nanocrystals or for conjugating them to a wide range of biomolecules of interest, and hence are increasingly used in a wide variety of biomedical research. In this study, we have evaluated various Qdot® nanocrystals (Qdot® 655 Carboxyl and PEGylated amine-functionalized variant) for in vivo toxicity in mice (CD-1/female; 12 or 18 / group; intravenous via tail vein; 0.333 -33.3 nM/Kg, 5 ml/Kg). LD50 values could not be estimated as maximally concentrated dosages were found to be non-lethal in all cases, but limiting values will be described. Additionally, no physiological, behavioral, or histological evidence of toxicological impact was observed within a wide range of practical working dosages, even following repeated dosing. Nevertheless, at very high doses, some toxicity was seen in the liver as determined by elevated liver enzymes (AST and ALT) after exposure to Qdot® 655 Carboxyl nanoparticles. Interestingly, studies on related test materials showed no toxic effect on the liver even at the highest dose strongly suggesting that particle composition is a strong determinant of toxicological behavior at these high dosages. These physiological changes were further evidenced by subtle histological changes found in the liver at the higher doses. Inflammatory changes were seen, predominantly around the portal veins. There was an increase in inflammation with some areas showing necrosis and changes suggesting early granuloma formation. In conclusion, clinical chemistry and histological changes were observed only for the highest doses, which are significantly higher than practical concentration for imaging in vivo in mice and the results of this study suggests that the Qdot® nanocrystals used in this study are not toxic at a useful concentration for in vivo imaging applications (approx. 20 pmol/animal).

PS 1198 EXPOSURE AND DISEASE SUSCEPTIBILITY DIFFERENCES IN U.S. AND SWEDISH POPULATIONS: IMPLICATIONS FOR ASSESSMENT OF RISKS FROM USE OF TOBACCO PRODUCTS.

C. Ward, G. Mariano and A. Santamaria. *Environ International, Arlington, VA.*

As the US looks to the Swedish experience with the smokeless tobacco product, snus, as a possible model for reducing harm from smoked tobacco, researchers have asked questions about the applicability of health effects data generated on the Swedish population to US populations. Numerous epidemiology and clinical studies exist on the potential population harms from snus in the Swedish population. We sought to understand whether the findings from these health studies can be expected in the US, even under the same or similar tobacco exposure scenarios, in terms of comparability of populations, on several levels of disease-related factors. First, standardized rates of tobacco-related diseases from the two countries were identified and compared. We also identified and compared rates of demographic, behavioral, and lifestyle factors that may influence susceptibility and disease risk. These include differences in diets, exercise and other lifestyle factors, and differences in potential influences from other environmental exposures, such as metals and persistent chemicals. A specific example is given for cadmium, a known nephrotoxicant. Cadmium exposures for smokers are known to be higher than those of nonsmokers, and the body burdens of cadmium in the Swedish population have decreased measurably over several decades, attributed to reductions in smoking rates. We discuss whether similar reductions in cadmium body burdens can be expected in the US from similar declines in smoking, and the implications for disease risk. In addition, we summarize known population differences between the US and Sweden in specific genetic polymorphisms and the potential influence of these differences on disease risk.

PS 1199 PULMONARY FUNCTION TESTING IN BOAT MANUFACTURER WORKERS.

G. T. Johnson, S. C. Harbison, J. D. McCluskey, P. Xu, L. Thomas, S. Goldman and R. D. Harbison. *Environmental and Occupational Health, University of South Florida, Tampa, FL.*

Styrene is widely used in boat manufacturing and uncontrolled exposure may lead to pulmonary function (PF) impairment in workers. This investigation used occupational health monitoring examination data to characterize PF in a population currently employed as boat manufacturer workers. PF tests for workers (n = 105) who required health examinations to ensure fitness for continued respirator use were compared to NHANES III Raw Spirometry subjects (n = 8,873) to determine

if abnormal PF was associated with employment as a boat manufacturer worker. Mean FVC and FEV1 values were determined and linear regression was used to evaluate the impact of boat manufacturer worker status on PF after adjusting for confounders. Workers produced statistically significant higher mean FVC and FEV1 values of 4.94 L (95%CI 4.75 - 5.13) and 3.86 L (95%CI 3.72 - 4.01) respectively compared to mean NHANES III subject values of 4.01 L (95%CI 3.98 - 4.02) and 3.20 L (95%CI 3.18-3.21). Stratification by age, height, and smoking status yielded similar results. Multivariate regression analysis demonstrated that significant predictors of FEV1 included age, height, gender, and pack-years of smoking (all p-values < 0.0001), but not boat manufacturer employment (p = 0.2023). Significant predictors of FVC included age, height, and gender (all p-values < 0.0001). Employment as a boat manufacturer worker was significantly associated with an increase in FVC (p < 0.0001). The moderate increase in PF observed in boat manufacturer workers is likely due to a combination of effective exposure controls in the workplace and the healthy worker effect.

PS 1200 CIRCULATING LEVELS OF PERSISTENT ORGANIC POLLUTANTS ASSOCIATE IN DIVERGENT WAYS TO FAT MASS IN HUMANS.

M. Rönn[1], L. Lind[1], B. van Bavel[2], S. Salihovic[2], K. Michaelsson[3] and M. P. Lind[1]. [1]*Department of Medical Sciences, Uppsala University, Uppsala, Sweden,* [2]*MTM Research Center, Örebro University, Örebro, Sweden and* [3]*Department of Surgical Sciences, Uppsala University, Uppsala, Sweden.*

Background: Environmental contaminants have recently been implicated in the pathogenesis of obesity. The present study aims to explore the relationship between Persistent Organic Pollutants (POPs) and fat mass in humans. Material and methods: In the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study, fat mass was determined by dual-energy X-ray absorptiometry (DXA) together with twenty-one POPs (including 16 polychlorinated biphenyl (PCB) congeners, 3 organochlorine (OC) pesticides, 1 brominated diphenyl ether (BDE47) and 1 dioxin) measured in plasma (high resolution chromatography coupled with high resolution mass spectrometry) collected at baseline in 890 participants aged 70 years. Results: Lipid-standardized plasma concentrations of Octa-chlorinated dioxin (OCDD), the PCBs 74, 99, 105 and 118 and the pesticides hexachlorobenzene (HCB), transnonachlordane (TNK) and dichlorodiphenyldichloroethylene (DDE), were all positively related to fat mass (p=0.03-0.0001). On the contrary, the PCBs 156, 157, 169, 170, 180, 189, 194, 206 and 209 were negatively related to fat mass (p=0.0001). Following adjustment for smoking, physical activity, education level, height, lean mass and gender these results remained significant (p=0.01-0.0001) except for PCB 74 and 99. For some of the negatively related PCBs the effects were more pronounced in women than in men. Conclusion: Plasma concentrations of some pesticides are positively related to fat mass, while divergent effects are seen for the PCBs. These results implicate a complex role of POPs in obesity.

PS 1201 CIRCULATING LEVELS OF PHTHALATES, BISPHENOL A, AND ABDOMINAL OBESITY.

M. P. Lind[1], M. Rönn[1], K. Michaelsson[2], D. A. Birkholz[3], L. Johansson[4], H. Ahlström[4] and L. Lind[5]. [1]*Department of Medical Sciences, Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden,* [2]*Department of Surgical Sciences, Uppsala University, Uppsala, Sweden,* [3]*Environmental Division, ALS, Edmonton, AB, Canada,* [4]*Department of Radiology, University Hospital, Uppsala, Sweden and* [5]*Department of Medical Sciences, Acute and Internal Medicine, Uppsala University, Uppsala, Sweden.*

BACKGROUND: The plastic manufacture substances phthalates and bisphenol A (BPA) has previously been suggested to be obesogens. Since abdominal obesity is more closely related to cardiovascular disease than peripheral obesity, we investigated the relationship between circulating levels of some phthalate metabolites, BPA and obesity measures obtained by Magnetic resonance imaging (MRI) and Dual energy X-ray absorptiometry (DXA). METHODS: In the population-based Prospective Investigation of the
Vasculature in Uppsala Seniors (PIVUS) study (1016 subjects all aged 70), serum levels of BPA and phthalate metabolites were measured by Isotope Dilution-High Performance Liquid Chromatography-Tandem Mass Spectrometry. Waist circumference and trunk fat mass by DXA were measured in 890 subjects, while subcutaneous and visceral abdominal fat were evaluated by MRI in 287 individuals. RESULTS: BPA and four phthalate metabolites (Monoisobutyl phthalate (MiBP), Monomethyl phthalate (MMP), Monoethyl phthalate (MEP), Mono-(2-ethylhexyl) phthalate (MEHP)) were detectable in almost all subjects. MiBP was related to waist circumference (p=0.013), trunk fat at DXA (p=0.004) and subcutaneous abdominal fat at MRI (p=0.001) in women, but not in men (p=0.05-0.003) for gender difference in association). BPA and the other 3 detectable phthalates did not relate to obesity measures. CONCLUSION: The phthalate metabolite MiBP is related to central obesity in women, but not in men.

PS 1202 ATHEROSCLEROSIS IS RELATED TO CIRCULATING LEVELS OF PERSISTENT ORGANIC POLLUTANTS (POPS) IN THE ELDERLY.

L. Lind[1], B. van Bavel[2], S. Salihovic[2] and M. P. Lind[3]. [1]*Acute and Internal Medicine, Department of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden,* [2]*MTM Research Centre, School of Science and Technology, Örebro University, Örebro, Sweden and* [3]*Occupational and Environmental Medicine, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.*

BACKGROUND: Increased circulating levels of persistent organic pollutants (POPs) have previously been associated with myocardial infarction. Since myocardial infarction is an atherosclerotic disease, we here investigated if levels of POPs are related to atherosclerosis. METHODS AND RESULTS: In the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (1016 subjects all aged 70), the prevalence of carotid artery plaques were recorded by ultrasound. The number of carotid arteries with plaques (0, 1 or 2) was recorded. Also the thickness (IMT) and grey scale (IM-GSM) of the intima-media complex were measured. Twenty-one different POPs including 14 PCBs, 5 pesticides, one dioxin and one brominated compound (BDE47) were analysed by high resolution chromatography coupled to high resolution mass spectrometry (HRGC/HRMS). Nine out of the PCBs (congeners 99, 153, 138, 156, 180,170, 194, 206 and 209) were related to the number of carotid arteries with plaques even after adjustment for gender, waist circumference, body mass index, fasting blood glucose, systolic and diastolic blood pressure, HDL and LDL-cholesterol, serum triglycerides, smoking, antihypertensive treatment and statin use (p=0.004-0.0001). High levels of the highly chlorinated PCBs (194,206 and 206) were associated with an echolucent IM-GSM (p<0.0001 after adjustment), while the relationships between POPs and IMT were modest. CONCLUSIONS: Circulating levels of PCBs were related to atherosclerotic plaques and the echogenicity of the intima-media complex independently of CV risk factors, including lipids. This might explain why POPs are related to myocardial infarction.

PS 1203 SERUM LEVELS OF POLYCHLORINATED BIPHENYLS IN MEXICAN WOMEN AND BREAST CANCER RISK.

R. Recio-Vega[1], V. Velasco-Rodriguez[2, 1], G. Ocampo-Gomez[1] and S. Hernandez-Gonzalez[1]. [1]*Environmental Health, University of Coahuila, Torreon, Coahuila, Coahuila, Mexico and* [2]*Clinical Epidemiology, IMSS, Torreon, Mexico.*
Sponsor: M. Cebrian-Garcia.

Background: Breast cancer is the most common invasive cancer in women worldwide and the primary cause of death in most developed and developing countries. Current established breast cancer risk factors explain only a fraction of the breast cancer cases diagnosed, and for this reason, other environmental factors have been studied during the past decade. Exposure to organochlorine compounds has been linked to an increased incidence of breast cancer, although not all data have been consistent. Objective: This case–control study was designed to investigate the association between polychlorinated biphenyl (PCB) levels and the risk of breast cancer. Material and Methods: We recruited 140 women from the General Hospitals 16 and 46 of the IMSS through 2006. The cases were 70 women newly diagnosed. We collected environmental and reproductive information through questionnaire. Blood samples were taken for serum levels of 20 PCB congeners measurement by the mean of GC-mass. Multivariate logistic methods were used to evaluate this association. Results: At least one PCB congener was detected in more than 77% of participants. The geometric mean for total serum PCB levels differ significantly in cases vs control (5.26 vs 3.33, ng/g, p=0.01). The risk of BC was positively associated with nine congeners (8,118,128,138,179,180,195,206, and 209) and with total PCB. When PCB were grouped by structure-activity relationships the risk of BC was positively associated with groups 2b (OR=1.90 95% IC 1.25-2.88), 3 (OR=1.81 95% IC 1.08-3.04), 4 (OR=1.57 95% IC 1.20-2.07) and total PCB (OR=1.09 95% IC 1.02-1.16). Among postmenopausal women, groups 1a, 2b, and 4 and total PCB were higher in cases. Conclusion: This study showed an association between serum PCB levels with increased risk of BC.

PS 1204 THE MODIFIER EFFECT OF ALAD 2 ON THE ASSOCIATION BETWEEN BLOOD LEAD AND HEMATOLOGIC OUTCOMES.

F. Scinicariello[1], B. A. Fowler[1], A. Yesupriya[2], M. Chang[2] and N. F. Dowling[2]. [1]*Division of Toxicology and Environmental Medicine, ATSDR/CDC, Atlanta, GA and* [2]*Office of Public Health Genomics, Centers for Disease Control and Prevention, Atlanta, GA.*

Objective: Several occupational studies have reported the ALAD 2 allele has a protective effect on certain hematological outcomes such as hemoglobin and erythrocyte zinc protoporphyrin in workers with elevated lead exposures. Method: We examined the genetic effects of ALAD polymorphisms on the relationship between

blood lead levels and hematopoietic biomarkers, such as erythrocyte protoporphyrin (EPP) and hemoglobin and hematocrit, using nationally representative data from the Third National Health and Nutrition Examination Survey (NHANES III) DNA bank, 1991-1994 (n=6016). Other covariates in the model were age, sex, race/ethnicity, hematocrit, serum transferrin saturation, serum ferritin. Results: In multiple linear regression models, at the highest blood lead quartile (3.7-52.9 μg/dL) ALAD was associated with log EPP. ALAD 2 carriers had statistically significant lower log EPP (β coeff=-0.10; SE= 0.02. p<0.001) compared to ALAD 1 homozygous individuals. Further analysis stratified by gender, showed that in the highest lead quartile, men that were ALAD 2 carriers had significant lower log EPP ((β coeff=-0.10; SE= 0.02. p<0.001) compared to ALAD 1 homozygous men. Women carrying the ALAD 2 allele had lower, but not significantly lower log EPP compared to ALAD 1 homozygous women. No significant statistical differences were found between ALAD 2 carriers and ALAD 1 homozygous individuals for hemoglobin and hematocrit. Conclusion: The ALAD 2 appears to modify the association between blood lead and EPP, and this significant protective association is found only at the highest blood lead quartile in males.

PS 1205 LEAD AND CHILDHOOD COGNITIVE DEFICITS: EXPLORING THE ROLE OF GENDER AND SOCIAL STRESS.

D. A. Cory-Slechta[1], K. Merchant-Borna[1] and E. Van Wjingaarden[2]. *[1]Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY and [2]Community and Preventive Medicine, University of Rochester Medical Center, Rochester, NY.*

Recent studies report neurodevelopmental and cognitive deficits in children below the Centers for Disease Control's (CDC) 10 μg/dL level of concern. The hypothalamic-pituitary-adrenal (HPA) axis interacts with frontocorticolimbic circuits that mediate cognition. Animal and human studies suggest that vulnerability to Pb-associated neurocognitive outcomes may be modified by psychological stress and gender. Understanding such potential effect modification is critical to elaborating mechanisms of neurotoxicity and to protect the most vulnerable members of the population. This study used data from the prospective longitudinal Rochester cohort study to evaluate potential gender and HPA axis modification of the adverse association between lifetime average blood lead levels (BLLs) and cognitive outcomes (IQ and measures of executive function) at 6 years of age by evaluating the role of maternal psychological stress as a surrogate for HPA axis function. Psychological stress was scored on the basis of responses to a set of 11 questions answered by mothers. As previously reported, lifetime average BLL was inversely associated with Full-Scale (p = 0.006) and Performance IQ (p = 0.002), but not Verbal IQ (p = 0.11). Although neither gender nor psychological stress significantly modified the association between BLL and cognition, there was a trend for a stronger BLL effect among children whose mothers report lower levels of social stress, while the dose-response of BLL with IQ was weak in those experiencing high levels of stress. Absence of this association at BLLs >10ug/dL could reflect the already lower IQ levels relative to those at BLLs < 10ug/dl resulting in a floor effect. The findings suggest the need for longitudinal studies to explicitly measure stressful elements of the social and physical environment as well as measuring biological markers of stress and its relationship to BLL.

PS 1206 OCCUPATIONAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS DECREASES SPERM QUALITY AND INDUCE DNA DAMAGE.

G. Ocampo-Gomez[1], E. Olivas-Calderon[1], R. Recio-Vega[1], G. Garcia-Arenas[2], R. Martinez-Salinas[3], J. Espinosa-Femat[2] and M. Gallegos-Arreola[4]. *[1]University of Coahuila, Coahuila, Mexico, [2]University of Durango, Gomez Palacio, Durango, Mexico, [3]University of San Luis Potosi, San Luis Potosi, Mexico and [4]University of Guadalajara, Guadalajara, Mexico.* Sponsor: M. Cebrian-Garcia.

During the last decade a growing incidence of genito-urinary abnormalities and decrease sperm quality in men have been reported. This increase has been linked to exposure to several environmental pollutants including the polycyclic aromatic hydrocarbons. Objective: To evaluate in Mexican men the association between urinary levels of 1-hidroxypirene (1-OHP)with the DNA damage, sperm quality and with the frequency of CYP1A1, GSST1 and GSTM1 polymorphism. Methods: A cross-sectional study was designed to evaluate the adverse effects of PHAs. A total of 71 subjects, 36 occupationally exposed and 35 unexposed were included in the study. Semen, urine samples and blood were collected with the aim to evaluate sperm quality by means of direct microscopy, 1-OHP urinary levels by HPLC with fluorescence detector, DNA damage by comet and micronuclei assays, and the frequency of CYP1A1, GSST1 and GSTM1 polymorphism by RLFP. Results: The mean age of exposed subjects was 30.2, meanwhile in controls it was 28.8. Mean time of occupational exposure was 10.05 years. Significant higher (p=0.001) 1-OHP levels were found in exposed subjects (0.65±0.91) than in controls (0.089±0.05). When the relationship between 1-OHP levels with sperm parameters was assessed, we found that sperm quality (sperm concentration/ml and total sperm count) was significantly lower in the exposed group. Four from eight DNA damage parameters (tail length, tail migration and tail moment, and the presence of micronuclei) were significantly higher in occupationally exposed subjects. No differences in the frequency of enzymes polymorphism were observed between studied groups. Conclusions: Exposure to PAHs decrease sperm quality and induce DNA damage. A negative association was found between 1-OHP levels with sperm concentration/ml and total sperm count, as well as, with the CYP1A1 variant m4.

PS 1207 THREE DECADES OF LOW-LEVEL EXPOSURE TO SPRAYED ORGANOPHOSPHATES: NEUROBEHAVIORAL OUTCOMES IN AGRICULTURAL WORKERS AND RESIDENTS OF RURAL COMMUNITIES.

Y. Finkelstein[1], A. Ophir[1, 2], M. Aschner[4], E. Doitsch[1], U. Wormser[2] and E. D. Richter[3]. *[1]Neurology and Toxicology Service and Unit, Shaare Zedek Medical Center, Jerusalem, Israel, [2]Institute of Drug Research, School of Pharmacy, Hebrew University, Jerusalem, Israel, [3]The Department of Occupational and Environmental Medicine, Faculty of Medicine, Hebrew University, Jerusalem, Israel and [4]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN.*

Previous studies (1987- 1991) established neurological effects of low-level long-term exposure to organophosphorous (OP) pesticides in adult agricultural workers and residents of rural communities in Northern Israel. The residents are, in all likelihood, exposed to pesticide drift due to the proximity of the communities to the fields. The original cohort test results showed in-season reversible neurobehavioral dysfunction. We are currently following up on the original cohorts. Testing methods employed include neurobehavioral status, cognition and task performance. The cognitive assessments include Trail Making Tests A and B, Digit Span (forward and backward) and Digit Symbol Tests. The preliminary results of the examinations of 98 adult members of the original cohort show cognitive changes. Trail Making Tests A and B were significantly poorer than those of the general population. The performance in Trail Making Test A was inversely correlated to the duration of environmental exposure (r=-0.231; α<0.01). Digit span test (forward and backward) was poor. Females performed better than males in all the cognitive tests. Data obtained provide important information on the health hazards of lengthy daily low-level exposures of agricultural workers and residents of rural communities to OP pesticides. Acknowledgements: This work was partially supported by grants from Environment Health Fund - No. RGA0903; Chief Scientist, Israel Ministry of Environmental Protection – No. 7-2-1; and Chief Scientist, Israel Ministry of Industry, Trade and Labor grant - No. 08-1-1028.

PS 1208 PRENATAL EXPOSURE TO DDT AND ITS EFFECT ON REPRODUCTIVE OUTCOME MEASURES.

I. Al-Saleh[1], I. Al-Doush[1], A. Alshabbaheen[1] and A. Rabbah[2]. *[1]Environmental Health Section, BMR Department, King Faisal Specialist Hospital & RC, Riyadh, Saudi Arabia and [2]King Khalid Hospital, Al-Kharj, Saudi Arabia.*

p,p'-DDT (bis[p-chlorophenyl]-1,1,1-trichloroethane) is a persistent organochlorinated insecticide that was banned in Saudi Arabia since 1982 for agriculture use, but it has been used occasionally in endemic areas with vector-borne diseases. We measured p,p′-DDE (the major metabolite of p,p′-DDT) in placenta, maternal and cord serum samples collected at delivery from 1578 women living in Al-Kharj, which undergoes sometimes to DDT spraying activities in response to sporadic leishmania cases. This study examined the possible effect of prenatal p,p'-DDT exposure on several reproductive outcome measures. p,p′-DDE was detected in 28% cord and 52% maternal serum, reflecting past exposure. Whereas the p,p′-DDT was only found in 0.5% cord and 1.4% maternal samples. As expected the DDE cord levels were 1.5 times lower than the maternal levels but both were significantly correlated (r=0.52) suggesting its transplacental transfer. p,p'-DDE and p,p′-DDT were detected in 84% and 99% of placental tissues with median values of 5.97μg/Kg dry wt. and 7.96μg/Kg dry wt. respectively. Placental p,p′-DDE/p,p′-DDT was 1.73 (range, 0-126.2). About 42% of women had a placental p,p′-DDE/p,p′-DDT ratio in the range of 1 to 5, which suggests recent and direct exposure to parent DDT. Our study showed a significant negative effect of maternal p,p'-DDE on head circumference (P=0.015), crown-heel length (P=0.011), birth

weight (P=0.016) and small for gestation age (P=0.008). As for the placental p,p'-DDE, an inverse association was seen with the head circumference (P=0), crown-heel length (P=0), birth height (P=0.002) and birth weight (P=0.007). Placental p,p'-DDT was also inversely related to crown-heel length (P=0), gestational age (P=0.018), placental thickness (P=0.003), placental diameter (P=0.023) and cord diameter (P=0). These results suggest that prenatal DDT exposure may have an impact on various measures of reproductive outcome, and further studies are urgently required to identify sources of this exposure in order to set preventive procedures.

PS 1209 TRENDS IN PARAOXONASE 1 ACTIVITY AND LIFESTYLE FACTORS IN A RURAL POPULATION OVER TIME.

L. Badtke[1], K. M. Kelly[2], K. Wang[3], J. A. Merchant[2] and G. Ludewig[1, 2]. *[1]Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA, [2]Occupational and Environmental Health, University of Iowa, Iowa City, IA and [3]Biostatistics, University of Iowa, Iowa City, IA.*

Paraoxonase 1 (PON1) is a glycoprotein capable of hydrolyzing many pesticides. There is substantial variation in serum PON1 activity among individuals within a population due to genetic, intrinsic and environmental factors. Low PON1 activity has been associated with increased risk for atherosclerosis and other disorders. It has been shown that PON1 levels appear to be lower in older populations, but no known study has measured serum PON1 activity in the same individuals over time. In a prospective population study ongoing since 1994 and now in its 3rd cycle of data/specimen collection, blood serum and questionnaire data are gathered roughly every 5 years from 499 rural Iowans ages 8-86 at the study onset. Since low PON1 activity increases the risk of pesticide toxicity, we were interested in the effects of lifestyle, diet, occupational exposure and age on PON1 activity. In this pilot study, the 3 serum samples from 31 females and 30 males were evaluated for PON1 activity using phenyl acetate and CMPA [4-(Chloromethyl)phenyl acetate] as substrates. Mixed model analyses (alpha = .05) did not significantly show the expected decrease in serum PON1 activity over time with either substrate. Body Mass Index, gender, location (farm/rural/town), and systolic and diastolic blood pressure did not significantly influence substrate levels, though gender, BMI and location showed a marginal influence. This population also demonstrated no significant changes in these factors over this 10-year span. This pilot study did not conclusively confirm the assumed age-related decrease in PON1 levels or changes in these lifestyle and health parameters. Increasing the number of participants and including more questionnaire data will allow us to identify risk factors for low PON1 status and allow us to counsel at-risk subpopulations about their individual pesticide vulnerability. (NIEHS P42ES013661 and U07/CCU706145 from CDC/NIOSH)

PS 1210 A CASE CONTROL STUDY OF CHRONIC MYELOMONOCYTIC LEUKEMIA IN SHANGHAI, CHINA.

S. A. Gross[1, 2], R. D. Irons[1, 3, 4], D. Galbraith[5] and D. Paustenbach[5]. *[1]Fudan-Cinpathogen Clinical and Molecular Research Center, Institutes of Biomedical Sciences, Fudan University, Shanghai, China, [2]ChemRisk, Boulder, Co., [3]School of Pharmacy, School of Medicine, University of Colorado, Denver, Denver, Co., [4]Cinpathogen, Boulder, CO and [5]ChemRisk, San Francisco, CA.*

We report the results of a hospital-based case-control study of 36 consecutive patients diagnosed with chronic myelomonocytic leukemia (CMML) over a 4-year period. Diagnoses were made by a single laboratory using 2001 WHO criteria. Subjects were age/gender matched to two controls and interviewed by questionnaire concerning previous disease, work histories and exposures to potential etiologic agents. Further sub-classification of CMML indicated 61% CMML-1, 22% CMML-2 with 16% not further defined. The frequency of clonal cytogenetic abnormalities in all CMML cases was 31%, with no specific pattern identified. Complex karyotypes were observed in five CMML cases, four of which involved chromosomes 5, 7 and or 8. Selected risk factors were evaluated and compared between the cases and control subjects. Five study subjects were determined to have some benzene exposure; a finding that was not statistically significant. There was no difference between cases and controls in exposure to other solvents or fuels. However, there was a difference in the proportion of cases exposed to organic phosphates versus control subjects, albeit not statistically significant (p = 0.07). The proportion of cases with a prior medical history of blood disorders was statistically significant (p = 0.02, CI -.008 to 0.230) and the proportion of cases with no prior medical disease was also statistically (p = 0.032, CI -0.351 to -0.038). None of the selected risk factors associated with non-occupational exposure to etiologic agents was significantly different between the study subjects. Our results indicate that chronic benzene exposure is not a risk factor for developing CMML.

PS 1211 NEW FOOD-BORNE HAZARD PARASITE TOXIN.

D. Irikura[2], M. Saito[1], Y. Yahata[3], Y. Sugita-Konishi[2] and Y. Kamata[2]. *[1]Kumagaya Meat Inspection Center Saitama Prefecture, Kumagaya, Saitama, Japan, [2]Division of Microbiology, National Institute of Health Sciences, Setagaya-Ku, Tokyo, Japan and [3]Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Shinjiku-Ku, Tokyo, Japan.*

In some parts of Japan, there is a traditional habit of eating raw equine flesh. Recently, a new food-borne disease, which is the major symptom of nausea and diarrhea, resulting from raw equine flesh, containing a Sarcocysis (S.) spp., which might be causative of the new food borne diseases. It has been known that S. hominis and S. suihominis are parasitic in bovine, swine, and human. However, the S. spp., being parasitic in equine is not a parasite for human. We already reported that intravenous injection of the bradyzoite of S.cruzi showed the toxicity such as diarrhea in rabbit. However, the influence of its toxicity to human has not been reported. In this study, we examined the relationship between the case of new food borne diseases and the toxicity of bradyzoite of S. spp., which was isolated from equine flesh. Results] The S. fayeri was found in equine flesh as a specimen, by microscopically and PCR. Experimental studies using the intestinal loop test and the intravenous injection in rabbit revealed that the bradyzoite of S. fayeri induced the entero-toxicity and bloody diarrhea or lethal. This toxicity was resistant to the freezing and thawing, by contrast, it was completely diminished by the pepsin treatment. Conclusion] In this study, our data demonstrated that the toxicity of the bradyzoite of S. fayeri possessed almost same feature of that of S. cruzi, which was isolated from bovine, indicating that it might be possible that a new protein toxin(s) exist in the bradyzoite of S. fayeri caused new food borne in human.

PS 1212 LEVELS OF EXHALED BREATH CONDENSATE PH AND FRACTIONAL EXHALED NITRIC OXIDE IN RETIRED COAL MINERS.

J. Lee, J. Shin, J. Lee, K. Lee, J. Kim and B. Choi. *Occupational Lung Diseases Institute, Comwel, Ansan, Republic of Korea.* Sponsor: I. Yu.

Inhaled inorganic dusts, such as coal, can cause inflammation and fibrosis in the lungs, known as pneumoconiosis. Diagnosis of pneumoconiosis depends on morphological changes by radiological findings and functional change by pulmonary function test (PFT). Unfortunately, current diagnostic findings are limited only to lung fibrosis, which is usually irreversibly progressive. Therefore, it is important that research on potential and prospective biomarkers for pneumoconiosis should be conducted prior to initiation of irreversible radiological or functional changes in the lungs. Analytical techniques using exhaled breath condensate (EBC) or exhaled gas are non-invasive methods for detection of various respiratory diseases. The objective of this study is to investigate the relationship between inflammatory biomarkers, such as EBC pH or fractional exhaled nitric oxide (FENO), and pneumoconiosis among 120 retired coal miners (41 controls and 79 pneumoconiosis patients). Levels of EBC pH and FENO did not show a statistically significant difference between the pneumoconiosis patient group and pneumoconiosis patients with small opacity classified by International Labor Organization (ILO) classification. The mean concentration of FENO in the low percentage FEV1 (< 80%) was lower than that in the high percentage (80% ≤) (p=0.023). The mean concentration of FENO in current smokers was lower than that in non smokers (never or past smokers) (p=0.027). Although there was no statistical significance, the levels of FENO in smokers tended to decrease, compared with non smokers, regardless of pneumoconiosis. In conclusion, there was no significant relationship between the level of EBC pH or FENO and radiological findings or PFT. The effects between exhaled biomarkers and pneumoconiosis progression, such as decreasing PFT and exacerbation of radiological findings, should be monitored.

PS 1213 *IN VITRO* TOXICITY CHARACTERIZATION OF WOOD COMBUSTION PARTICLES.

D. R. Dietrich[1], B. Pieterse[2], B. van der Burg[2] and S. Gauggel[1]. *[1]Human & Environmental Toxicology, University of Konstanz, Konstanz, Germany and [2]BioDetection Systems BV, Amsterdam, Netherlands.*

Exposures to particulate matter (PM) from wood combustion furnaces have the potential to induce respiratory tract diseases in humans. Amongst the main factors contributing to human respiratory tract toxicity are the physicochemical characteristics of the PM as well as the loading of the particles with carcinogenic compounds e.g., polycyclic aromatic hydrocarbons (PAHs). PAH mixtures induce xenobiotic metabolism via activation of the aryl hydrocarbon receptor (AhR) and downstream expression of metabolizing enzymes. Estimation of PAH toxicity exclusively based on chemical analyses appears insufficient as these analyses are routinely restricted to

a limited subset of PAHs (16 US-EPA PAHs). Therefore, additional potential toxicity assessment via in vitro assays as well as mechanistic studies e.g., assessing PAH availability to the cells and understanding the activation and/or suppression of signal transduction, appears warranted. The aim of this study was to assess PAH toxicity via chemical PAH analysis, a specific in vitro PAH transactivation assay and metabolism studies. Two different PM samples were analyzed (TD-GC-MS/MS) for the presence of 16 PAHs. PAH-induced toxic potency was determined with the PAH CALUX transactivation assay. The PAH CALUX system suggested the presence of a toxic potency several fold above that indicated by chemical analysis. In addition, human type II alveolar epithelial cells (A-549) were exposed to different PM concentrations and Benzo(a)pyrene (B[a]P) as a positive control. PM as well as B[a]P exposure resulted in a higher CYP1A1 mRNA expression than in controls. CYP1A1 enzyme activity was confirmed via a luminogenic assay. CYP1A1 enzyme activity was inhibited at high PM and B[a]P concentrations and assumed to result from increased expression of AhRR, a repressor of the AhR signaling cascade. Consequently understanding the status of both the AhR and AhRR appear important in order to interpret the potential toxicity of PMs and B[a]P in human alveolar cell systems.

PS 1214 SULFUR EMISSIONS FROM CHINESE DRYWALL IN CHAMBERS AND HOMES.

J. Matheson[1], M. Babich[1], T. Thomas[1], K. Hatlelid[1], J. Recht[1], R. Maddalena[2], M. Apte[2], J. McCarthy[3], B. Baker[3], J. Allen[3], D. MacIntosh[3] and L. Saltzman[1]. *[1]U.S. Consumer Product Safety Commission, Bethesda, MD, [2]LBNL, Berkeley, CA and [3]EH&E, Needham, MA.*

The CPSC has received 3600 consumer complaints reporting health effects and metal corrosion in homes built/renovated in 2001-2008. Reported health effects include: migraines, nosebleeds, irritated eyes, dyspnea, cough, and sinus problems. Affected homeowners often note the homes have an offensive odor. Certain drywall imported from China (CDW) was implicated as the probable cause of reported corrosion; but other home materials have not been ruled out as contributory factors. Chamber testing of domestic and imported drywall emissions and in-home air sampling are part of a multi-track test program to evaluate the relationship between drywall emissions, material degradation, and reported symptoms. Chamber testing on 30 drywall samples was performed at LBNL. Emissions from preconditioned samples were measured in 10.5 L chambers at a ventilation rate of 0.5 L/min at 25°C and 50% relative humidity. Four methods were used for emission sampling and analysis depending on the target chemical class. A new method, solid phase micro extraction, was developed to measure reactive volatile sulfur gases. The majority of emissions consisted of H_2S and SO_2; total sulfur emission factors ranged from 0.3 to 209 μg/m^2/h. Higher sulfur gas emissions were observed from CDW made in 2005-2006. EH&E's investigation in 51 homes included source characterization using FTIR and XRF; measurements of indoor environment including gases, ventilation rate, temperature and relative humidity; and copper and silver corrosion rates. Compared to control homes, complaint homes had significantly higher H_2S levels (0.82 μg/m^3 vs ⟨LOD) and rates of copper sulfide (476 vs ⟨LOD A/30d) and silver sulfide corrosion (1472 vs 389 A/30d). A consistent finding of elevated sulfur emissions associated with CDW was observed in the chamber and in-home testing. The home sulfur gas levels were below known irritant levels; however, it is possible that additive or synergistic effects of these and other compounds could potentially cause irritant effects to consumers.

PS 1215 EXPOSURE MODEL FOR INDIVIDUALS (EMI) IN HUMAN HEALTH STUDIES: PREDICTING RESIDENTIAL INDOOR EXPOSURES TO FINE AIRBORNE PARTICLES.

M. Breen[1], M. Breen[1, 2], B. Schultz[1], T. Long[1], R. Williams[1], A. Vette[1] and R. Devlin[1]. *[1]U.S. EPA, Research Triangle Park, NC and [2]North Carolina State University, Raleigh, NC.*

In many epidemiological studies, particulate matter (PM) is associated with increased risk for adverse cardiopulmonary events. Due to cost and participant burden of indoor and personal measurements, health studies often estimate exposures using ambient measurements. However, ambient levels do not necessarily reflect personal exposures since indoor levels can differ from ambient levels, and people spend considerable time indoors. To reduce this exposure error, which adds uncertainty to risk estimates, we are developing an exposure model for individuals (EMI) in cohort health studies. A critical aspect of EMI is estimation of the ambient contribution to concentrations within individual homes, where people spend most of their time. A mass-balance indoor air quality (IAQ) model was linked to an air exchange rate (AER) model to predict ambient-generated indoor $PM_{2.5}$ mass from ambient concentrations, meteorology, and questionnaire data on housing characteristics and operation. First, AER model predictions were compared to 642 daily AER measurements across 31 detached homes in central North Carolina. For individual model predicted and measured AER, median absolute difference was 40% (0.17 hr^{-1}). Second, IAQ model was evaluated with concurrent daily measurements of ambient and residential indoor-outdoor $PM_{2.5}$ mass. Using cross validation, model predictions were compared to measurements of ambient-generated indoor $PM_{2.5}$, which were derived from sulfate (outdoor tracer of $PM_{2.5}$). For individual model predictions and measurements, median absolute difference was 24% (2.2 μg/m^3). This study demonstrates the ability of EMI to predict residential indoor $PM_{2.5}$ of ambient origin in support of developing exposure metrics for health studies.

PS 1216 EXPOSURE TO PHTHALATES AMONG PREMENSTRUAL GIRLS FROM RURAL AND URBAN, GHARBIAH, EGYPT.

J. Colacino[1, 2], A. Soliman[2, 3], M. Nahar[1], A. Hablas[4], I. Seifeldin[4], A. Van Zomeren-Dohm[1], L. S. Rozek[1] and D. C. Dolinoy[1]. *[1]Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, [2]Center for Global Health, University of Michigan, Ann Arbor, MI, [3]Epidemiology, University of Michigan, Ann Arbor, MI and [4]Tanta Cancer Center, Gharbiah, Egypt.*

Phthalates have been identified as endocrine active compounds associated with developmental and reproductive toxicity. Phthalate exposure in premenstrual Egyptian females, a potentially vulnerable population, remains unknown. The objective of this study was to quantify phthalate exposure in premenstrual females from rural and urban areas of the Gharbiah province of Egypt as well as describe potential routes of exposure. We collected one spot urine sample from 60 premenstrual females, 30 each from urban and rural areas, as well as anthropometrics and questionnaire data regarding cooking, cosmetic use, and food storage behaviors. Urinary concentrations of 11 phthalate metabolites were quantified by isotope dilution tandem mass spectrometry at the National Center for Environmental Health of the Centers for Disease Control and Prevention. Urinary concentrations of diethyl phthalate and di(2-ethylhexyl) phthalate metabolites measured in this study were similar to those measured in age-matched US girls in the National Health and Nutrition Examination Survey 2003-2006. Median mono-benzyl phthalate concentrations were higher in US girls (31.1 ng/mL) compared to Egyptians (1.6 ng/mL). In contrast, median levels of mono-isobutyl phthalate were higher in Egyptians (21.1 ng/mL) compared to US girls (7.5 ng/mL). We found no significant difference in concentrations of phthalate metabolites based on the urban or rural status of the Egyptian girls. In principle components analysis, storage of food in plastic was found to be associated with phthalate body burden (p=0.036), suggesting that dietary intake is likely an important route of phthalate exposure in both urban and rural Egyptians. These findings suggest that phthalate exposure in developing countries may be of equal concern as in the US.

PS 1217 BLOOD ANALYSIS OF SWEDISH FIRE VICTIMS 1992-2009 SUGGEST HYDROGEN CYANIDE AS AN IMPORTANT CAUSE OF DEATH.

K. Stamyr[1], G. Thelander[2], L. Ernstgård[1] and G. Johanson[1]. *[1]Work Environment Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and [2]Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden.*

BACKGROUND For people caught in fires, or fire fighters entering fires, a major threat is posed by toxic fumes. 60-80% of all deaths related to fire have been attributed to such fumes. Carbon monoxide (CO) is a major component and is commonly thought to be the major cause of most fire-related fatalities. However, hydrogen cyanide (HCN) is also formed, especially during combustion of nitrogen-containing materials such as polyurethane foam, a material increasingly encountered in indoor environments. The exact contribution of HCN to fire-related fatalities is unknown (Stamyr et al. Toxicol. Lett. 2008). AIM The study was undertaken to investigate the impact of HCN in relation to CO as a cause of intoxication and death in fire victims. METHOD Swedish data on all fire victims in the period 1992-2009 were collected from two nationwide forensic databases (ToxBase and RättsBase). Among the 1300 victims, 500 had been analyzed for both carboxyhemoglobin (COHb) and HCN in blood. Nearly half of the victims had lethal COHb levels (>50% COHb). Further, 7% had HCN levels above 2 μg/g (an indication of lethal or life-threatening intoxication), 30% had levels above 1 μg/g and over 50% had levels above 0.5 μg/g (an indication of significant HCN exposure). HCN continues to be eliminated from blood also after death (McAllister et al. J. Anal. Toxicol. 2008) and the exact time lapse from death to sampling and analysis is unknown for these blood samples. Thus, measured HCN is probably a serious underestimate of true blood levels at the time of death. CONCLUSION Overall, these Swedish forensic data suggest that HCN may be an equally or even more im-

portant cause of death, compared to CO. ACKNOWLEDGMENT The study was supported by Ångpanneföreningen and the Swedish National Board of Health and Welfare.

PS 1218 DERMAL AND INHALATION EXPOSURE OF EGYPTIAN COTTON WORKERS DURING CHLORPYRIFOS APPLICATIONS.

R. Fenske[1], F. M. Farahat[2], K. Galvin[1], J. R. Olson[3], D. S. Rohlman[4] and W. K. Anger[4]. [1]*University of Washington, Seattle, WA,* [2]*Menoufia University, Shebin Elkom, Egypt,* [3]*State Univesity of New York at Buffalo, Buffalo, NY and* [4]*Oregon Health & Science University, Portland, OR.*

Concerns about health risks to Egyptian cotton workers who apply organophosphorus (OP) pesticides, including chlorpyrifos (CPF) prompted study of inhalation and dermal exposures to chlorpyrifos, as well as measurement of the CPF urinary metabolite 3,5,6-trichloro-2-pyridinol (TCP). Sampling was conducted during the summer of 2009 at cotton fields in the Egyptian Governorate of Menoufia for 12 workers, four from each job category: applicators who used backpack sprayers; technicians who assisted applicators in the fields; and engineers who supervised from outside the field. Personal air samples were collected with OVS sampling tubes at 1.0 LPM. Dermal patches were attached to worker clothing or skin at six locations. Geometric mean (GM) air concentrations by job category were compared using the Welch Anova and Bonferroni test. Mean shift duration was 3.5 hr (SD=0.24 hr). The GM CPF air concentrations were significantly different ($p<0.001$) between the three job categories; applicator values (GM=44.5 $\mu g/m^3$) were significantly greater ($p<0.000$) than both engineer (GM=5.1 $\mu g/m^3$) and technician values (GM=8.2 $\mu g/m^3$). The dermal CPF mean loading rates were highest on the upper legs for all job categories (58, 2.9 and 0.33 $\mu g/cm^2/hr$ for applicators, technicians, and engineers, respectively). These rates were generally lower than those measured in a previous (2007) study. The highest TCP concentration in urine (699 $\mu g/g$ creatinine) was measured in an applicator two days after the spray day. The high CPF loads on the upper legs point to the need for protection of this body region as a first step to minimize exposure. Study results were presented to focus groups of Ministry of Agriculture officials and cotton workers. The groups used the data to select interventions that use low cost local materials. These interventions are now part of an intervention evaluation study. (NIH R01 ES016308; Anger & Lein, MPIs)

PS 1219 CIGARETTE SMOKING AND GENETIC POLYMORPHISMS IN THE ARYL HYDROCARBON RECEPTOR MAY BE ASSOCIATED WITH MENOPAUSAL HOT FLASHES.

A. Ziv-Gal[1], L. Gallicchio[2, 3], S. R. Miller[3], H. Zacur[3] and J. A. Flaws[1]. [1]*Comparative Biosciences, University of Illinois, Urbana, IL,* [2]*The Prevention and Research Center, Mercy Medical Center, Baltimore, MD and* [3]*School of Medicine, Johns Hopkins University, Baltimore, MD.*

Cigarette smoking has been associated with an increased risk of menopausal hot flashes (HFs). The aryl hydrocarbon receptor (AHR) plays a central role in mediating the toxic effects of chemicals in cigarette smoke. Interestingly, single nucleotide genetic polymorphisms (SNPs) in the *AHR* and *CYP1B1* (an AHR regulated gene) have also been associated with HFs. Though HFs are the most common condition reported during the menopausal transition, their etiology is unknown. Thus, we hypothesized that women who carry a SNP in the *AHR* gene (R554K) and smoke cigarettes have a higher risk of experiencing more frequent HFs compared to women who do not carry the SNP and smoke cigarettes, and that the same association between the SNP and HFs is not present among women who 'never smoked cigarettes'. To test our hypotheses, 634 women were recruited from Baltimore and its surrounding counties to participate in a cross-sectional study of midlife women. The women completed detailed questionnaires and donated blood samples. DNA was extracted and genotyped using allele specific polymerase chain reactions, followed by agarose gel electrophoresis. Associations between the SNP and frequency of HFs were statistically analyzed by logistic regression for women who 'ever smoked cigarettes' and those who 'never smoked cigarettes' separately. The results indicate that women who 'ever smoked cigarettes' and carry the SNP have an increased risk of experiencing daily HFs compared to women who 'ever smoked cigarettes' and do not carry the SNP (odds ratio: 2.7; 95% confidence interval: 1.1-6.8; adjusted for age, race, body mass index and estradiol levels). Among women who 'never smoked cigarettes', carrying the SNP was not associated with frequency of HFs. Collectively, these data suggest that a combination of the SNP in the *AHR* and cigarette smoking is associated with an increased risk of daily HFs. Supported by NIH R01 AG 18400.

PS 1220 EVALUATION OF NEUROLOGICAL, CARDIAC, AND ENDOCRINE EFFECTS OF CARBON DISULFIDE EXPOSURE IN HUMANS.

S. B. Diwan, J. C. Lipscomb, J. Whalan, N. Keshava and C. Shoaf. *Office of Research and Development, NCEA-W, U.S. EPA, Washington, DC.* Sponsor: B. Diwan.

We reviewed the available epidemiological evidence from a Japanese occupational cohort to inform an evaluation of neurotoxic, cardiac and endocrine effects in humans exposed to carbon disulfide (CS2). This prospective cohort study was conducted in male workers exposed to CS2 in 11 Japanese rayon manufacturing plants, with 6-year follow up. A total of 432 CS2-exposed workers and 402 age-matched non-exposed referent workers were examined in the baseline study; 750 of these had follow-up examinations. Mean duration exposure was 19.3± 8.1 years for all CS2 workers. Height, weight, body mass index, and weekly alcohol intake were similar in CS2 exposed and non-exposed groups. Twice-yearly measurements of 2-thiothiazolidine-4-carboxylic acid in the urine and 8-hour Time-Weighted-Average (TWA) levels served as measures of exposure. In the baseline study and cohort follow-up, effects of CS2 on cardiovascular measures (blood pressure, arterial stiffness, electrocardiogram, brain Magnetic Resonance Imaging (MRI), opthalmography etc.), nervous system function (motor and sensory nerve conduction velocities, digital span etc.) and endocrine effects (FSH, ACTH, TSH, T3, T4, etc.) were assessed. Follow-up of this cohort focused on brain MRI results, cardiovascular function, neurological effects, and endocrine function. Air monitoring revealed an 8-hour TWA of 3.36 ppm during baseline, and a level of 5±1.8 ppm during the follow-up period. Significant increases in retinal aneurysm and neurological effects were observed in workers exposed to CS2 level of 3.36 ppm and above. Finding of neurological effects in occupationally exposed workers is consistent with what is known about cross-linking of axonal proteins as part of the mode of neurotoxicity for CS2. Based on these findings we determined that 3.36-ppm (10.5 mg/m3) is the lowest level among all the Lowest-Observed-Adverse-Effect-Levels (LOAELs) reported in the literature for an 8-hr TWA occupational exposure to CS2.

PS 1221 OCURRENCE OF PERFLUORINATED COMPOUNDS IN WATER SUPPLY NETWORKS OF CATALONIA, SPAIN.

M. Nadal[1], J. L. Domingo[1], I. Ericson Jogsten[2], G. Perelló[1], E. Bigas[3], B. van Bavel[2] and X. Lleberia[3]. [1]*Toxicology, Rovira i Virgili University, Reus, Spain,* [2]*Örebro University, Örebro, Sweden and* [3]*Catalan Public Health Agency, Department of Health, Barcelona, Spain.*

Since 2007, a wide surveillance program aimed at analyzing the levels of a number of perfluorinated compounds (PFCs) in environmental, biological and food samples is being carried out in Catalonia (Spain). Because of the potential implications on public health, special attention has been paid to drinking water. In winter 2009, samples were collected in 10 water supply networks of Catalonia. In each network, three sampling points were considered: a) at the own source, b) in a point after water treatment to make it safe drinking, and c) in public fountains. Analysis of 15 different PFCs in the 30 samples was performed using an Acquity UPLC coupled to a Quattro Premier XE MS/MS. PFOA and PFOS showed the highest mean concentrations (2.40 and 1.81 ng/L, respectively). In agreement with recent studies from our laboratory, water samples from Barcelona Province, an area characterized by a strong industrial activity, showed the highest concentrations of PFCs. Although no significant differences were observed in the PFC levels according to the respective sampling point within the supply network, slight lower concentrations were noted after treatment of the waters. It would indicate that the current systems allow a certain reduction of PFCs (elimination percentage ranged between 3 and 53%) contained in water. Based on the levels here detected, the mean exposure to PFOS and PFOA from drinking water for the adult population of Catalonia would be 3.9 and 4.8 ng/day, respectively. These values are clearly lower than the maximum thresholds established by the European Food Safety Authority. Therefore, the current PFC burden in drinking water should not pose health risks for the consumers.

PS 1222 AGRICULTURAL USE OF MANEB INCREASES SOIL MN LEVELS IN AN EXPERIMENTAL STUDY: IMPLICATIONS FOR ENVIRONMENTAL MN EXPOSURE.

T. Cobban[1], J. van den Berg[1], A. Bradman[2], B. Eskenazi[2] and D. R. Smith[1]. [1]*University of California, Santa Cruz, CA and* [2]*University of California, Berkeley, CA.*

Rationale: Elevated environmental exposure to manganese (Mn) is associated with neurobehavioral deficits in children and adults. The fungicide Maneb is ~20% Mn by weight and over 140,000 kg are applied annually in the Salinas Valley, CA. Our preliminary studies (Guiner et al., SOT abst) found that Mn levels in carpet dust

were correlated with proximity to agricultural uses of Maneb. Here we tested the hypothesis that Maneb use increases agricultural soil Mn levels, and thereby may serve as a source of increased house dust Mn loadings in agricultural communities. Procedures: Agricultural soil was aliquoted in 6x6x8 cm containers and treated with 1) Control (DI water vehicle), 2) Annual Equivalent Maneb application (Maneb 80 WP), or 3) 5x Annual Equivalent Maneb (n=5/trt). Maneb was applied every other day over 6 days (3 applications total) to achieve soil surface application levels equivalent to annual or 5x annual use. Plots were maintained in full sunlight and watered daily. Soil was then sieved to <150 or <75 µm fractions and aliquots of each were sequentially extracted for Mn using a modified BCR protocol using 0.11 M CH3COOH, 0.5 M HONH2-HCl, or 7.5 M HNO3. Leachate fractions were analyzed for Mn Al, and Fe by ICP-OES.
Results: Maneb application at the annual or 5x annual equivalent increased soil Mn levels by ~2-fold or 5-fold, respectively, in all three extraction fractions and in both size fractions. Forty – 50% of total extracted Mn was contained within the acetic acid fraction, while extracted Mn normalized to naturally occurring Al (as Mn/Al ratio) was 30 – 50-fold greater in the acetic acid fraction compared to the other two. Conclusions: These data indicate that ~50% of soil/dust Mn is chemically and biologically labile, and that Maneb application significantly increases soil/dust Mn levels. This result supports concern of increased environmental dust Mn exposure in communities proximal to agricultural Maneb use.

PS 1223 ENVIRONMENTAL AND HEALTH EFFECTS RESULTING FROM HEAVY METALS EXPOSURE IN LUANDA SOIL FROM ANGOLA, AFRICA.

M. E. Gomes, C. G. Yedjou and P. B. Tchounwou. *Biology, Jackson State University, Jackson, MS.*

Heavy metals are natural constituents of the Earth's crust. They are stable and cannot be degraded or destroyed, and therefore they tend to accumulate in soils and sediments. In trace amounts, heavy metals are necessary to maintain good health. However, at high concentrations they become hazardous by causing acute or chronic toxicity. Acute exposure to heavy metals can be associated with neurological damage, behavioral disorders, loss of memory, and other negative health effects. Each metal has a unique features and physic-chemical properties that confer to its specific toxicological mechanisms of action. Hence, the goal of the present study was to monitor and assess the level of heavy metals in soil samples collected in the city of Luanda located in Angola, Africa. To achieve to goal, the Niton handheld XRF analyzer was used to measure the levels of various heavy metals in soil samples collected from Luanda (the largest city in Angola). The level of each heavy metal detected was compared to Environmental Protection Agency (EPA) standards and regulatory guidelines. Among the soil samples tested, we detected several metals with means ± SDs values of 20.9 ± 10.0 ppm arsenic, 50361.98 ± 387.51 ppm calcium, 63.69 ± 14.48 ppm copper, 22085.74 ± 238.13 ppm iron, 270.70 ± 12.52 ppm lead, 330.95 ± 48.14 ppm magnesium, 14614.94 ± 352.25 ppm potassium, 1674.66 ± 41.58 rubidium, 176.03 ± 5.14 ppm strontium, 1376.30 ± 345.52 sulfur, 3550.54 ± 113.12 ppm titanium, 254.16 ± 15.68 ppm zinc, 818.42 ± 11.23 ppm zirconium in Luanda soil. We observed that the concentrations of each metal vary significantly from one site to another. Many metals detected above the EPA standards and regulatory guidelines. Based on the EPA standards and regulatory guidelines, we conclude Luanda soils in Angola are contaminated with heavy metals, and this environment is not safe for children activities.

PS 1224 CIGARETTE SMOKING IN CHINA AND THE U.S.: AN EVALUATION USING THE CHINA HEALTH AND NUTRITION SURVEY AND THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY.

S. J. Kathman[1], K. M. Marano[1], Z. S. Naufal[1], H. Gan[1], J. Xie[2], H. Liu[2], X. Li[2], P. Shang[2], C. D. Garner[1] and C. L. Wilson[1]. *[1]RJ Reynolds Tobacco Co., Winston Salem, NC and [2]Zhengzhou Tobacco Research Institute, Zhengzhou, China.*

To evaluate behavioral (i.e., consumption) and demographic (i.e., age, sex, body weight) differences of current cigarette smokers in China and the United States (US), data from the China Health and Nutrition Survey (CHNS) and the National Health and Nutrition Examination Survey (NHANES) were analyzed and compared. CHNS and NHANES are public-use data sets. CHNS is a longitudinal sample from nine provinces in China and NHANES is a survey representative of the US civilian, non-institutionalized population. For this analysis, the adult survey questionnaire for CHNS 2006 and the NHANES 2005-2006 household questionnaire for adults were used. Average age of current smokers was similar in China (49 years) and the US (42 years), whereas average body weight was different, i.e., 63.8 kg in China and 80.2 kg in the US. Although the overall prevalence of current smokers in China and US was similar (27.2 vs. 24.0%), prevalence by sex was different: 53.3% for males and 3.5% for females in China and 27.7% for males and 20.6% for females in the US. In order to determine if CHNS data are nationally representative, results were compared with previously reported prevalence and daily cigarette consumption values. The values from CHNS were consistent with other reports of current smoking in China (approximately 50-60% for males and 2-4% for females). CHNS average daily cigarette consumption (16.6) was also consistent with previously published values for China (approximately 14-17). Similarly, in the US, average consumption was 15.6 cigarettes per day. In summary, current cigarette smokers in China and the US differed by sex and body weight. However, age and daily cigarette consumption were similar. These data allow for a better characterization of cigarette smoking exposure in the US and China, which is necessary for a proper understanding of cigarette smoking-related health risks in the US and Chinese populations.

PS 1225 AN APPROACH TO "CHEMILESS OFFICE" WHICH AIMS TO PREVENT SICK-BUILDING SYNDROME BY IMPROVING THE OFFICE INDOOR AIR.

H. Nakaoka[1, 2], E. Todaka[3, 1], M. Hanazato[2, 4], H. Seto[5] and C. Mori[1, 2, 3]. *[1]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Chiba, Japan, [2]Center for Preventive Medical Science, Chiba University, Chiba, Chiba, Japan, [3]Center for Environment, Health and Field Sciences, Chiba University, Chiba, Chiba, Japan, [4]Department of Architecture, Graduate School of Engineering, Chiba University, Chiba, Chiba, Japan and [5]Tokyo Kenbikyo-in Foundation, Tachikawa, Tokyo, Japan.*

There is increasing number of people suffering from "Sick-building Syndrome" at office environment in Japan. The purpose of this study was to create "Chemiless Office" which aims to prevent Sick-building Syndrome by improving indoor air at office space and show the specifications of the prototype. At first, the emission rates of chemicals from the wall paper and floor carpet were measured by small chamber test method. The emission rates of each material were 35.2µg/m2/h, 146µg/m2/h respectively and the concentration level of volatile organic compounds (VOCs) in indoor air when these materials were used in the office were estimated low enough. However the first model office which was made of those materials in the reinforced concrete building in April 2009, 3 out of 6 office staffs claimed symptoms of nausea. Then the indoor air of the office was analyzed and the result showed that the total VOCs (TVOC) was 667.8µg/m3, and isododecane was detected as 583µg/m3. Isododecane was first detected indoor air. It became clear that the source of isododecane was the adhesive of pedestals to support the raised floor. The floor was remodeled not using adhesive, the concentration level of isododecane was lowered to 2.2µg/m3 and then TVOC was lowered to 267µg/m3 in August 2010. Isododecane is one of the chemicals which are not suspected as causing chemicals of Sick-building Syndrome. To prevent Sick-building Syndrome, it is necessary to create office environment in a way the TVOC is low enough.

PS 1226 ARE PEAK WORKPLACE EXPOSURES ASSOCIATED WITH STYRENE NEUROTOXICITY?

N. El Majidi, A. Vyskocil, R. Thuot, C. Beaudry, G. Charest-Tardif, R. Tardif, F. Gagnon, B. Ska, A. Turcot and C. Viau. *Université de Montréal, Montréal, QC, Canada.*

The manufacture of fibreglass reinforced plastic products may give rise to substantial peak exposures to styrene. Such exposure pattern needs further consideration in terms of styrene neurotoxicity. The aim of this study was to verify if the neurotoxic effects of styrene are related to long-term peak exposures in workers at levels respecting the Quebec occupational exposure limits (8-h-TWA of 213 mg/m3 and 15-minutes average of 426 mg/m3). A total of 104 workers (51 women, 53 men) were recruited in fibreglass- reinforced plastic industry in Quebec. Their average inhaled styrene concentration was used to separate them in three exposure categories: "Low" (< 42.6 mg/m3), "Medium" (42.6 – < 213 mg/m3), and "High" (> 213 mg/m3). The exposed groups were then subdivided in two sub-groups: "with peaks" or "without peaks". Average styrene exposure was measured by personal passive dosimetry during the work shift on three consecutive days (Tuesday, Wednesday and Thursday). On one day, exposed workers were continuously monitored during the various tasks using a portable gas chromatograph taking samples every minute. The neurotoxicity of styrene was evaluated using a battery of internationally recognized sensory and neurophysiological tests. The results show that average 8-h TWA exposure in the visited industries ranged up to 520 mg/m3. Important peak concentrations were identified and their average duration ranged between 12.5 and 22.1% of the work shift. In many cases, the peak concentrations largely exceeded the short-term limit value. Irritation of eyes, nose and throat was significantly more frequent in the High compared to the Medium or the Low-ex-

posure groups. However, the different exposure scenarios involving average styrene exposure or peak exposures did not significantly alter the performance to any test. Several factors could have influenced our results: definition of peaks, statistical power or short exposure time.

PS 1227 UTILITY OF C6-GLIOMA CELLS FOR EXPLORATORY RISK ASSESSMENT OF MIXTURES OF INSECTICIDES.

D. M. Romero[1], M. L. Kotler[1] and M. J. Wolansky[1, 2]. [1]*Biological Chemistry (Toxicology), ARG NRC / University of Buenos Aires School of Science, Buenos Aires, Argentina and* [2]*ARG National Agency for Scientific and Technological Promotion, Buenos Aires, Argentina.*

Low levels of organophosphate (OP) and pyrethroid (PYR) insecticides may co-occur in real environments and food products. Considering each compound independently, these residue loadings may be considered safe for humans. However, there is a consensus on the need of considering joint neurotoxicity data to improve the accuracy of health risk estimates for these chemicals. We use a C6-glioma cell culture as an exploratory in vitro system derived from rat nervous system aimed to identify toxicologically relevant OP-PYR mixtures that may require cumulative risk analysis efforts in in vivo models. First, 4 OPs (chlorpyrifos; acephate; methidathion; diazinon) and 4 PYRs (type I: bifenthrin, tefluthrin; type II: α-cypermethrin, deltamethrin) were examined in single-compound assays carried out in C6-cells after 4-48 hr exposure to each compound (0.1-250 μM). Threshold doses for cell viability status were determined using optical microscopy and MTT assays. We generated time- and dose-effect data, and ED15 values were computed from assays conducted at t=24 hr. In general, micromolar exposures produced dose-related alterations in cell viability at 24-48 hr. ED15s were 5-100 μM for PYRs and 5-30 μM for OPs. Second, we evaluated the effects of each insecticide on nucleus morphology using Hoechst 33258 staining. We found dose-related increases in anomalous nuclei shapes from the low μM range up. Last, we examined a mixture of equitoxic levels of the 4 PYRs (1X mixture = Σ ED15) using the MTT assay. This type-I/II mixture produced a dose-related, dual joint action at 24 hr: while addition of individual effects was observed after exposure to the 1X mixture, dose-addition was apparent at a 0.1X dose, i.e., well below the single-compound thresholds for mitochondrial impact. We plan to evaluate other mixtures of insecticides in order to select toxicologically relevant combinations for in vivo testing of cumulative neurotoxicity.

PS 1228 BARIUM INTAKE IN THE CANARY ISLANDS: A TOTAL DIET STUDY.

C. Rubio, D. González-Weller, J. Gutiérrez and A. Hardisson. *Toxicology, University of La Laguna, La Laguna, Spain.* Sponsor: A. Anadon.

Barium (Ba) is a divalent metal widely distributed in nature. Commercial uses make it a potential food contaminant. The main sources of exposure for humans are food consumption, water consumption, and inhalation. Ba appears in many common foods at concentrations ranging from 0.21 to 11.0 mg/kg. Chronic exposure to this element is often associated with toxic effects on the cardiovascular system of mammals. Objectives: To quantify the concentration of Ba in 22 food groups as categorized by the Canary Islands Nutrition Survey (ENCA, 2000) and to estimate the total Ba intake in various segments of the population and the general population. Material and Methods: A total of 440 samples were analyzed to determine the Ba levels in various food groups. Ba was determined by Optical Emission Spectrometry with Inductively Coupled Plasma (ICP-OES). Results: The average concentration of Ba in the analyzed food groups was 0.65 mg/kg, implying an intake of 0.69 mg/day of Ba in the adult population of the Canary Islands. All groups had detectable Ba levels. Concentration levels ranged from 0.04 ± 0,04 mg/L in water to 5.21 ± 2,12 mg/kg in nuts. The food consumption patterns of various genders and age groups were considered along with the Ba levels in the 22 food groups. The highest estimated dietary intake levels were observed in the 45-54 age group, for both men and women. Conclusions: This study demonstrates the need to assess, understand and monitor both the levels of metals in various food groups and the dietary intake of the population, in order to determine a safe intake level.

PS 1229 HOUSE DUST-MEDIATED PAH EXPOSURES RELATED TO SEALED PARKING LOTS MAY EXCEED DIETARY INTAKES IN CHILDREN.

E. S. Williams[1], B. J. Mahler[2] and P. C. Van Metre[2]. [1]*Center for Reservoir and Aquatic Systems Research, Baylor University, Waco, TX and* [2]*U.S. Geologic Survey, Austin, TX.*

Most assessments have indicated that dietary ingestion is the primary route of exposure to carcinogenic polycyclic aromatic hydrocarbons (B2 PAHs; the seven PAH compounds classified as probable human carcinogens by the EPA) for adults and children. Studies have estimated that dietary B2 PAH intakes in children are 24.8 ng/kg/day or less, generally larger those associated with inhalation or non-dietary ingestion of soil and house dust. Recent research has indicated that the use of coal tar-based (CT) sealants on parking lots may be associated with increased concentrations of B2 PAHs in settled house dust (SHD) in nearby living spaces (compared with unsealed asphalt lots). We hypothesized that, for young children living in residences adjacent to CT-sealed parking lots, exposures to B2 PAHs through ingestion of SHD might surmount dietary intake as the primary route of exposure. Using default exposure assumptions of 17.4 kg body weight and 10 and 50 mg/day for dust ingestion rates, doses of 25.01 and 125.03 ng/kg/day B2 PAH were calculated for a 4 year old child living in a residence next to a CT-sealed parking lot, compared to 1.73 and 8.65 ng/kg/day for children living near unsealed asphalt parking lots. According to this screening-level exposure model, children in living spaces adjacent to parking lots sealed with CT-based products may be exposed to B2 PAHs primarily through ingestion of SHD.

PS 1230 DIRECT COMPARISON OF DRIED BLOOD SPOT (DBS) ANALYSIS TO PLASMA AND WHOLE BLOOD ANALYSIS IN TOXICOKINETIC STUDIES OF RATS.

L. Patrone[1], G. Liu[2], H. M. Snapp[2], A. Batog[1], J. Valentine[1], R. S. Mangipudy[1], A. Tymiak[2], Q. C. Ji[2] and M. E. Arnold[2]. [1]*Drug Safety Evaluation, Bristol-Myers Squibb, New Brunswick, NJ and* [2]*Bioanalytical Sciences, Bristol-Myers Squibb, Lawrenceville, NJ.*

Dried blood spot (DBS) analysis is a method gaining recognition for obtaining drug exposure data. To evaluate the fidelity of DBS analysis compared to the accepted practice of plasma analysis, comparison of the data obtained by these methods is necessary. The primary objective of this presentation is to demonstrate how BMS is exploring DBS toxicokinetic applications in rats by directly comparing plasma to DBS from the same animal. In this study, 2 compounds were evaluated; BMS-X and Omeprazole (a marketed proton pump inhibitor). Twelve rats were evaluated for each compound, and 4 rats were used as controls to determine contamination potential. 300 ul blood was drawn from each rat. Blood was spotted directly from the collection tube onto the DBS cards in four 15 ul aliquots. The remaining blood was centrifuged to obtain plasma. The ratio of blood to plasma concentration was calculated and the "predicted" blood concentrations were obtained by applying the ratio to the plasma concentrations. Toxicokinetic curves were constructed by averaging the concentrations at each time point. Results obtained from DBS, blood, and plasma were consistent while liquid whole blood analysis was slightly more variable. Advantages of DBS include no need to centrifuge samples, easy sample storage and shipment, small sample volume, and easier sample preparation for analysis. Challenges include difficulties in assay development for hydrophilic compounds. Carry-over and cross contamination during sample analysis was not an issue. However, additional precaution is needed to prevent this when an assay has a low lower limit of quantitation (LLOQ).

PS 1231 DRIED BLOOD SPOT (DBS): A POTENTIAL APPROACH TO MONITORING DRUG EXPOSURE IN RAT FOETUSES.

S. Cinelli[1], F. Pastori[2], R. Cicalese[3], S. Villa[4] and G. Oberto[1]. [1]*Scientific Direction, Research Toxicology Centre RTC, Pomezia - Rome, Italy,* [2]*Analytical Chemistry, Research Toxicology Centre, Pomezia - Rome, Italy,* [3]*Toxicology, Research Toxicology Centre, Pomezia - Rome, Italy and* [4]*Operative Direction, Research Toxicology Centre, Pomezia- Rome, Italy.* Sponsor: J. Brightwell.

Dried Blood Spot (DBS) is a promising technique to reduce blood sample volume during non-clinical and clinical studies. For this reason the technique has been extensively applied in the human paediatric population and could be used to monitor foetal drug exposure during non-clinical reprotoxicity studies. For this experiment oxacarbazepine was orally administered to pregnant rats and, following validation, the DBS technique was used to evaluate placental barrier crossing, testing blood from both dams and foetuses. Oxacarbazepine is an antiepileptic drug which is known to cross the placental barrier. Analytical methods were validated using Ultra Performance Liquid Chromatography and Tandem Mass Spectrometry (UPLC-MS/MS) on plasma samples, applying the traditional extraction procedure, and on whole blood spotted on DBS cards. Oxacarbazepine was administered to pregnant Sprague Dawley female rats by oral gavage during the gestation period. Blood samples were collected at necropsy from dams and foetuses. Blood taken from dams was used to generate both plasma and DBS samples to compare performance of the two procedures. Blood samples taken from foetuses were sufficient only to apply the DBS technique. The results of the described experiment, demonstrating placental transfer of oxacarbazepine, confirmed the applicability of DBS technique to evaluate drug levels in dams and litters during reprotoxicity studies.

PS 1232 COMPARATIVE KINETICS USING DRIED BLOOD SPOT TECHNIQUE (DBS) IN THE CYNOMOLGUS MONKEY.

S. Grote-Wessels[1], M. Niehoff[1], W. Wilbois[1], T. Pfaff[2], H. Fischer[3], A. Welbers[2] and W. Mueller[1]. [1]*Covance Laboratories GmbH, Muenster, Germany,* [2]*AiCuris GmbH & Co.KG, Wuppertal, Germany and* [3]*A&M Labor, Bergheim, Germany.* Sponsor: G. Weinbauer.

The generation of highly reliable TK exposure data is vital to success of a toxicology programme. Within preclinical studies in small animals like juvenile cynomolgus monkeys (Macaca fascicularis) or the common marmoset (Callithrix jacchus) it can be a challenge to provide sufficient blood for the preparation of plasma or serum whilst staying within acceptable volumes. Blood sampling strategies such as DBS employs the use of small quantities of blood while providing powerful analytical capabilities. In addition to ethical benefits and depending on characteristics of each compound DBS offers the potential to overcome problems associated with the traditional plasma/serum bioanalysis. We present a study within which the plasma bioanalysis was compared to results generated by DBS for AIC090027, a HCMV terminase inhibitor. The bioanalytical method was validated over the range of 1 to 1000 ng/mL AIC090027 in blood of the adult cynomolgus monkey by combining extraction from the spotted card with quantification by LC/MS. The method displayed good accuracy and precision over the concentration range and was successfully employed in a preclinical study following oral administration of AIC090027 to cynomolgus monkeys. Overall, in vivo data generated with DBS samples showed that Tmax and AUC (area under curve) following daily administration of 60 and 120 mg/kg (5 male animals/group) were comparable to plasma analysis. In addition, DBS results were in good agreement with historical in vitro plasma data when allowance was made for the plasma blood partition ratio. For DBS samples of the high dose group (240 mg/kg) compound concentrations were in excess of those previously observed values and data has to be interpreted with caution. Nevertheless this comparison DBS and plasma data demonstrates the potential of this technique to provide reliable data for systemic exposure apart from the matrix used, blood or plasma.

PS 1233 CHARACTERISATION OF BIOLOGICALLY AVAILABLE WOOD COMBUSTION PARTICULATES IN CELL CULTURE MEDIUM.

S. Gauggel[1], C. Derreza-Greeven[1], J. Wimmer[1], M. Wingfield[2] and D. R. Dietrich[1]. [1]*Human & Environmental Toxicology, University of Konstanz, Konstanz, Germany and* [2]*Malvern Instruments GmbH, Herrenberg, Germany.*

Combustion of wood is one of the major sources of particulate matter (PM). Exposures to this type of PM have the potential to induce respiratory tract diseases in humans. The toxicity of the particles appears to be the result of their physicochemical characteristics. Many in vitro studies address the particle toxicity by investigating cytotoxic, inflammatory and genotoxic effects as well as their mechanisms via submerse exposure. However, few if any submerse in vitro studies, including those at the air liquid interface, characterised the actual particle fraction and characteristics within the culture medium that finally induced the observed response. Indeed, the fact that availability of particles, suspended in liquids, may be dramatically altered due to particle agglomeration with a subsequent influence on their toxicity was largely ignored. This study looks at the influence on the particle size distribution on the bioavailability to cells. The particle size distribution and agglomeration status of ambient PM (from wood furnaces), engineered nanoparticles (inert latex particles) and amorphous and crystalline quartz of known size in cell culture medium and water was determined using scanning Electron Microscopy (SEM) and light scattering techniques such as dynamic light scattering and laser diffraction (Zetasizer, Nanosight, Mastersizer). The analyses of the engineered latex particles, amorphous quartz and crystalline quartz samples confirmed the size defined by the manufacturer. While engineered latex particles (≤ 100 nm) and amorphous quartz appeared to agglomerate in medium, this was less so for crystalline quartz. In comparison measurement of complex mixtures e.g., wood combustion PM in media demonstrated the presence of very small particles and a small amount of large particles, formed through agglomeration as in the case of crystalline quartz.

PS 1234 DIESEL EXHAUST PARTICLE TOXICITY TO HUMAN LUNG EPITHELIAL CELL LINE: SINGLE CELL EXPOSURE ASSESSMENT WITH MORPHOLOGICAL AND BIOPHYSICAL ANALYSIS.

G. D. McEwen[1], Y. Wu[1], R. A. Coulombe[2] and A. Zhou[1]. [1]*Biological Engineering, Utah State University, Logan, UT and* [2]*Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT.*

Single cell level investigation of cell membrane properties under toxic exposure is important in basic understanding of the roles of cell biophysical properties (e.g., cell adhesion and biomechanics) in cytotoxicity and genotoxicity. In this study, electric cell-substrate impedance sensing (ECIS), Raman microspectroscopy (RM), and atomic force microscopy (AFM) were applied to *in vitro* monitor the changes of cell morphology, cellular bio-components and biomechanical and nano-architectural properties of viable human lung epithelial (A549) cells exposed to diesel exhaust particles (DEP) over time. ECIS results indicated that DEP treatment (125, 250, 500 μg/ml) on an adherent cellular monolayer, decreased cellular viability; whereas, DEP treatment (5, 17, 50, 125, 250, and 500 μg/ml) prior to attachment and spreading result in a decrease of adhesion and cellular proliferation rates. For two single cell analyses, RM revealed changes of cellular bio-components based on characteristic Raman band intensity at 785 cm^{-1} (DNA), 939 cm^{-1} (carbohydrates), 1006 cm^{-1} (proteins), and 1092 cm^{-1} (lipids) for both DEP treated and untreated A549 cells; AFM results indicated that DEP treatments induced variations in the cellular membrane surface hydrophobicity, a significant decrease in the membrane surface adhesion force over treatment time, and some alteration in cell elasticity. In conclusion, this study demonstrated a (sub-)cellular toxicity assessment via multi-instrumental approach to help further reveal and understand potential links among morphological, biochemical, and cyto-architectural/biomechanical changes of human lung carcinoma epithelial cells induced by DEP.

PS 1235 SUPERCOARSE WORLD TRADE CENTER PARTICLE EFFECTS ON RAT BRONCHIAL AIRWAY FUNCTION.

B. Garrett, J. Vaughan, M. Cohen and L. Chen. *Environmental Health Science, New York University, New York, NY.*

The World Trade Center (WTC) collapse on 9-11-01 released copious amounts of particles into the air. In the first few days after the disaster, First Responders (police, firefighters, civilians) - many without respiratory protection - worked for hours under strenuous conditions that resulted in heavy oral breathing. In the period since the disaster, responders have complained of various health issues ranging from chronic cough to debilitating ailments. Using a WTC dust mixture bearing primarily (>85%) supercoarse (10-53 μm) particles collected on-site over the first 3 days post-collapse, inhalation exposures in rodent models were performed. Here, exposures were followed by methacholine (Mch) challenge to ascertain if these mostly particles affected bronchial airway activity. To mimic an average Responder exposure of ≈4 hr/d at Ground Zero in the period of maximal dust levels, 8-wk-old F344 rats were sedated with isoflurane (ISO) and then dosed via intratracheal inhalation with WTC dust particles for 2 hr/d for 3 days. To verify that use of long sedation in a supine position was not affecting airway reactivity, a subset of rats were placed on their backs and maintained at 1.5% ISO for 2 hr/d for 3 d. At days 1, 3, 7, and 14 after the final ISO exposure, the rats underwent aerosol Mch challenge and response analyses via whole body plethsmography. Air-exposed controls were challenged in parallel. For the WTC dusts, a set of rats underwent exposure to a single high dose (100 mg/m3) of particles (MMAD=25 μm) for 2 hr/d for 3 days. The rats then underwent Mch challenge at days 1, 3, 7, and 14 post exposure. Rats dosed solely with ISO did not exhibit any changes in bronchial airway reactivity in comparison to air controls. Alterations in airway reactivity were noted in rats dosed with the WTC dust mixture; these rats exhibited significant differences in reaction time to lower doses of Mch in comparison with controls. This indicated that the large WTC particles caused significant changes in airway function (specifically hyperreactivity) and potentially caused other damage.

PS 1236 ASSESSMENT OF PUBLIC HEALTH RISKS ASSOCIATED WITH NAPHTHALENE ENTERING RESIDENCES AND COMMERCIAL SPACE FROM VAPOR INTRUSION AT MGP SITES.

R. B. DeHate[1], B. Skelly[1], G. T. Johnson[2] and R. D. Harbison[2]. [1]*GEI Consultants, Tampa, FL and* [2]*Environmental and Occupational Health, University of South Florida, Tampa, FL.*

Naphthalene is a constituent of coal tar and often a contaminant found at former manufactured gas plants (MGP). Development at these sites has resulted in residential and commercial areas with potential exposures from vapor intrusion.

Naphthalene is routinely analyzed in soil vapor intrusion assessments for properties overlying and surrounding former MGP sites. Naphthalene exposure from vapor intrusion may be a public health risk. The purpose of this study was to evaluate three sites located in the northeast United States to determine the frequency of naphthalene detection in indoor air. A total of 76 properties were included in the study. A total of 469 indoor air samples were analyzed for naphthalene by EPA Method TO-14A or TO-15. The properties were sampled on 157 occasions and some properties sampled up to 7 times. At residential properties indoor air samples were collected in basements and crawl spaces, and in occupied areas such as living rooms and bedrooms. At commercial properties indoor air samples were collected in basements, sump rooms, storage closets and common areas such as lobbies, classrooms, and offices. A typical sample collection included indoor air testing in the basement area, living room (with a duplicate sample) and a bedroom. These data were then used to evaluate exposure and assess public health risks. Naphthalene concentrations ranged from 0.26 to 51 ug/ cubic meter of air. Only 3 of the 472 indoor air samples detected naphthalene above the ninety-fifth percentile background value of 12 ug/ cubic meter of air. These 3 detections could be explained by sources of naphthalene not associated with vapor intrusion. Naphthalene was detected in less than 1% of the indoor samples. Naphthalene vapor intrusion was not a public health risk among the 76 properties built on or near the former MGP sites.

PS 1237 EARLY LIFE MANGANESE EXPOSURE ESTIMATED USING DECIDUOUS TOOTH DENTINE AS A BIOMARKER.

M. Arora[1, 2], A. Bradman[3], K. Harley[3], M. Vedar[3], J. Chevrier[3], N. Holland[3], B. Eskenazi[3] and D. R. Smith[1]. *[1]University of California, Santa Cruz, CA, [2]University of Sydney, Sydney, NSW, Australia and [3]University of California, Berkeley, CA.*

Rationale: The absence of a suitable retrospective biomarker of Mn exposure during the crucial prenatal and early childhood periods, when susceptibility to adverse effects of Mn may be heightened, remains a major obstacle for epidemiologic studies on health effects of early environmental Mn exposure. Procedures: The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study recruited pregnant women before 20 weeks gestation and collected environmental and biological matrices, including shed deciduous teeth, prospectively over a period of approximately 8 years. Tooth Mn (as Mn:Ca ratios) was measured at 40 locations in pre- and postnatally formed regions of 18 deciduous teeth using laser ablation-ICPMS. Mn levels in teeth, shed at a mean age of 6.8 years, were compared with concentrations in maternal blood and urine collected at the 26th gestational week, cord (fetal) blood, maternal and child urine collected six months postnatally, and Mn concentrations and loading in house dust collected at the 26th gestational week. Results: Mn levels in both pre- and postnatal regions of dentine (as Mn:Ca area under the curve) were significantly associated with Mn loading in house dust (µg Mn/m2 floor area) (Spearman's ρ-prenatal=0.57, p=0.01 and ρ-postnatal=0.48, p=0.05, n=18). Mn concentrations in cord blood were significantly associated with Mn levels in ablation spots in dentine on either side of the neonatal line corresponding to the perinatal period ($p<0.05$). There was a significant association between maternal blood Mn level at 26 wks gestation and cord blood Mn level at birth (ρ=0.88, p=0.004,n=8), but not between any other maternal or child biomarker (blood, urine) and floor dust Mn levels. Mn levels in enamel did not show a clear association with any of the Mn biomarkers. Conclusions: Results suggest that Mn measurements in dentine of deciduous teeth may reflect exposure during the perinatal periods.

PS 1238 VALIDATION OF HAIR MANGANESE AS A EXPOSURE BIOMARKER: ANALYTICAL CONSIDERATIONS AND ASSOCIATIONS WITH OTHER EXPOSURE MARKERS.

D. R. Smith[1], T. Jursa[1], F. Donna[4], N. Zimmerman[2], E. Hoffman[3], R. Wright[3] and R. Lucchini[4]. *[1]University of California, Santa Cruz, CA, [2]Purdue University, West Lafayette, IN, [3]Harvard University, Boston, MA and [4]University of Brescia, Brescia, Brescia, Italy.*

Rationale: The absence of well-validated biomarkers of manganese (Mn) exposure in children remains a major obstacle for human studies of Mn toxicity. We evaluated the relationship between hair Mn levels with other biomarkers (blood, urine) and environmental Mn measures among Italian children. We compared residents of a community impacted by Mn contamination from ferroalloy plant activity to a control community. Because of hair's susceptibility to contamination, we also determined the suitability of several cleaning/processing methods for removing external Mn from dust and water contamination. Procedures: Children 11-13 yrs were enrolled from the ferroalloy plant-impacted region (Valcamonica, n=150) and the control community (Garda Lake, n=150). Blood, urine, and hair were collected to assess internal Mn dose. To assess concurrent (air) and past (soil) Mn exposure, personal air and soil samples were collected. Results: Hair Mn correlated with Mn in soil (ρ=0.316, $p<0.001$) and trended towards correlation with air Mn (ρ=0.215, p=0.061). No correlation was found between hair Mn and Mn in blood/urine, or between blood/urine Mn and soil/air Mn. Within the lab, hair treated with dust under controlled conditions showed a 15-30-fold increase in Mn, but rigorous cleaning was effective in removing this external contamination. Treatment of hair with Mn-contaminated water (70 or 700 ppb) increased Mn levels by 4-30-fold, though, rigorous cleaning was not effective in removing external contamination from water. Conclusion: These data suggest that hair Mn correlates with environmental Mn exposure in children, while blood and urine Mn do not. Furthermore, rigorous cleaning of hair is sufficient to remove external Mn contamination from dust, but not from contamination from water containing elevated Mn.

PS 1239 THE VALUE OF INFORMATION FROM PROVISIONAL PBPK MODELS IN SUPPORT OF BIOMONITORING: THE CASE OF ATRAZINE.

Y. Tan[1], R. Goldsmith[1], C. Daniel[1], X. Zhang[3], R. Tornero-Velez[1], J. Johnson[2] and C. Dary[2]. *[1]U.S. EPA, Durham, NC, [2]U.S. EPA, Las Vegas, NV and [3]General Dynamic Information Technology, Henderson, NV.*

Atrazine (ATZ) is a widely applied triazine herbicide used on corn, sorghum, and sugarcane in the U.S. ATZ and its chlorinated metabolites are found in both drinking water sources and other bodies of water. To evaluate environmental exposures to ATZ, the Center for Disease Control and Prevention (CDC) measured atrazine mercapturate (AM), an ATZ metabolite, in the 2001-2002 National Health and Nutrition Examination Survey (NHANES). Levels of AM were generally below the detection limit of 0.3 µg/L. A later study found that by measuring urinary AM alone, CDC had been underestimating ATZ exposures since desethylatrazine (DEA) and diaminochlorotriazine (DACT), not AM, are the predominant urinary metabolites. Moreover, the variation in proportion of various ATZ metabolites among persons was large. To better understand and explore the quantitative relationship among different biomarkers and how they impact estimation of ATZ exposure, a provisional human physiologically based pharmacokinetic (PBPK) model was developed by scaling up an existing rat model. In addition, the model was modified to incorporate additional metabolites. Parameters for this model were obtained either from existing models or estimated using quantitative structure activity relationships. This provisional human model was then applied to investigate possible time course behaviors of different metabolites under various exposure scenarios, such as exposure to both ATZ and metabolites or drinking water exposures with different concentrations of ATZ. Furthermore, the model was used to estimate ATZ concentrations to which herbicide applicators have been exposed based on their urinary AM levels, as well as ATZ concentrations associated with the detection limit of AM in NHANES. Several data gaps are discussed in biomonitoring efforts in the context of the value of provisional models in selecting optimal biomarkers for human health risk assessment. *This abstract has been cleared by the US-EPA but solely expresses the view of the authors.*

PS 1240 A TOXICOKINETIC MODEL TO ASSESS EXPOSURE TO FOLPET FUNGICIDE FROM URINARY BIOMARKERS.

R. Heredia Ortiz[1], A. Berthet[1, 2] and M. Bouchard[1]. *[1]Department of Environmental and Occupational Health, University of Montreal, Montreal, QC, Canada and [2]Institute for Work and Health, Lausanne University, Lausanne, Switzerland.*

Folpet is one of the most widely employed fungicides in agriculture. It is typically used in the culture of vegetables, fruits and ornamental plants. Once absorbed in the human body, it has been found to be very reactive, especially in acid conditions. According to various in vitro and in vivo experiments in animals, Folpet is first fractioned at the N-S link when in contact with aqueous solutions and thiol groups. From this non-enzymatic process a phthalimide (PI) molecule is formed, which may be used as a biomarker of exposure, along with the short-lived thiophosgene. We have built a human toxicokinetic model to account for the biotransformation of Folpet into PI and its subsequent excretion while accounting for other non-monitored metabolites. The mathematical parameters of the model were determined accordingly from best-fits to the time courses of PI in blood and urine of five volunteers administered orally 1 mg/kg and dermally 10 mg/kg of Folpet. In both cases, the mean elimination half-life of PI from the body (either through faeces, urine or

metabolism) was found to be 31.6 h. The average final fractions of administered dose recovered in urine as PI were 0.025% and 0.002%, for oral and dermal administration, respectively after 96 h. According to the model, when orally administered, PI rapidly hydrolyzes to phthalamic and phthalic acids such that only 0.04% of the PI found in the gastrointestinal tract is absorbed into the blood stream. Likewise, after dermal application, model predicts that only 7.4% of the applied Folpet dose crosses the epidermis. In the model, the PI initial metabolite of Folpet is formed in the dermis and further metabolized prior to reaching systemic circulation, such that only 0.125% of PI formed at the site-of-entry reaches systemic blood. Our mathematical model is in accordance with both measures of blood (R^2=0.57 for dermal and R^2=0.66 for oral) and urine (R^2=0.98 for dermal and R^2=0.99 for oral).

PS 1241 EVALUATION OF CARDIOVASCULAR DISEASE BIOMARKERS IN ADULT TOBACCO CONSUMERS: A COMPARISON BETWEEN THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY AND A SINGLE SITE CROSS-SECTIONAL STUDY.

K. M. Marano, S. J. Kathman, B. A. Jones, B. G. Brown and M. F. Borgerding. *RJ Reynolds Tobacco Co., Winston Salem, NC.* Sponsor: C. Garner.

Cardiovascular disease (CVD) physiological and biochemical markers of potential harm were compared in adult cigarette smokers, smokeless tobacco (chewing tobacco and snuff) consumers, and non-consumers of tobacco using data from the National Health and Nutrition Examination Survey (NHANES). A similar evaluation was then conducted in a subset of NHANES, which included male cigarette smokers, snuff consumers, and non-consumers of tobacco ages 26-49. These two NHANES analyses were then compared with results from a single site, cross-sectional study of healthy U.S. male exclusive cigarette smokers, exclusive moist snuff consumers, and never consumers of tobacco ages 26-49. In both NHANES analyses and the cross-sectional study, median values for all markers were within normal clinical reference ranges. However, statistically significant differences were seen in some comparisons between the three tobacco consumption categories, which were generally consistent across the three data sets. Most differences were seen in cigarette smokers compared with non-consumers of tobacco (increases in white blood cells, neutrophils, monocytes, lymphocytes, fibrinogen, and C-reactive protein, and decreases in folate) and in cigarette smokers compared with smokeless tobacco consumers (increases in white blood cells, monocytes, and lymphocytes). In smokeless tobacco consumers compared with non-consumers of tobacco, only folate was statistically significantly different (decreased) in all analyses. In general, findings were consistent with the available published data of CVD biomarkers in smokeless tobacco consumers compared with non-consumers. Results additionally support previous reports of statistically significant differences in certain biomarkers of CVD in cigarette smokers and non-smokers.

PS 1242 AN ANALYSIS OF PERFLUOROCHEMICAL CONCENTRATIONS IN AMERICAN RED CROSS ADULT BLOOD DONORS, 2000 TO 2010.

G. W. Olsen[1], D. C. Mair[2], C. C. Lange[3], M. E. Ellefson[3] and W. K. Reagen[3]. *[1]Medical, 3M Company, St. Paul, MN, [2]North Central Region, American Red Cross, St. Paul, MN and [3]Environmental Laboratory, 3M, St. Paul, MN.*

In 2000, the 3M Company voluntarily began phase out production of perfluorooctanesulfonyl-based products that were used as surfactants, paper and packaging treatments and surface protectants after perfluorooctanesulfonate (PFOS, C8F17SO3-) was reported in human populations and the environment. The majority of the manufacture phase-out was accomplished by the end of 2000. Between 2000-2001 and 2006 an approximate 60% decline in PFOS concentrations was observed across 6 American Red Cross blood donation centers representing Boston, MA., Charlotte, N.C., Hagerstown, MD., Los Angeles, CA., Minneapolis-St. Paul, MN., and Portland, OR. An approximate 25% decline was observed for perfluorooctanoate (PFOA, C7F15COO-). The present study extends these trend statistics an additional four years as the length of time since the phase-out is nearly twice the reported mean half-life of serum elimination for PFOS of approximately 5 years. In the summer of 2010, 600 plasma samples were collected from 300 male and 300 female American Red Cross anonymous blood donors between the ages of 20-69 at the same six locations. Sample extraction is accomplished using protein precipitation. The final eluates are analyzed for PFOS, perfluroohexanesulfoante (PFHxS, C6F13SO3-), perfluorobutanesulfonate (PFBS, C4F9SO3-), and a homologous series of perfluorocarboxylates from perfluorobutyrate (PFBA, C3F7COO-) through perfluorododecanoate (PFDA, C11F23COO-), using high-performance liquid chromatography interfaced with a triple-quadrupole mass spectrometer. Quantification is accomplished using matrix-matched extracted calibration curves in combination with isotopically-labeled internal standards and surrogates.

PS 1243 PLASTICIZER MIGRATION FROM TOYS AND CHILD CARE ARTICLES.

M. A. Dreyfus and M. A. Babich. *U.S. Consumer Product Safety Commission, Bethesda, MD.*

Dialkyl phthalates, including diisononyl phthalate (DINP), have been used as plasticizers in children's products made from polyvinyl chloride (PVC), such as teethers and toys. Children may be exposed when handling or mouthing PVC products because plasticizers are not covalently bound. The Consumer Product Safety Improvement Act of 2008 (CPSIA) prohibited the use of certain phthalates in child care articles and children's toys. Thus, manufacturers have changed to other plasticizers or non-PVC plastics. However, the potential health risks of alternative plasticizers have not been evaluated. CPSC staff purchased 63 children's products comprising 129 components before the phthalate regulations took effect. Plastics identified by FTIR included PVC, polypropylene, polyethylene, and ABS. Of the 129 components tested, 38 were PVC. Plasticizers identified by GC-MS in the 38 PVC components included acetyltributyl citrate (ATBC) (20); di(2-ethylhexyl) terephthalate (DEHT) (14); 1,2-cyclohexanedicarboxylic acid diisononyl ester (DINCH) (13); 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB) (9); di(2 ethyhexyl) phthalate (DEHP) (1); and DINP (1). Most tested components contained multiple plasticizers. Total plasticizer concentrations ranged from 2.5 to 58%. Migration rates were measured by the Joint Research Centre method to estimate oral exposure when the articles are mouthed. Migration rates (micrograms/10 cm^2/min) were: ATBC (range 0.8 to >15); TXIB (0.9—11.3), DINCH (0.4—7.3); and DEHT (0.1—3.6). Migration rates did not correlate well with plasticizer concentration. Wipe sampling was used to estimate the amount of plasticizer that could be transferred to skin. Plasticizer release ranged from 490 (ATBC) to 32,000 (DINP) micrograms per 100 cm^2. This study was performed to assist a Chronic Hazard Advisory Panel (CHAP) convened by the CPSC, as directed in the CPSIA, "to study the effects on children's health of all phthalates and phthalate alternatives as used in children's toys and child care articles." *This work has not been reviewed or approved by, and does not necessarily represent the views of, the Commission.*

PS 1244 RESIDENTIAL EXPOSURE MODELING FOR OZONE-GENERATING AIR CLEANERS.

M. A. Babich, K. R. Carlson and T. A. Thomas. *U.S. Consumer Product Safety Commission, Bethesda, MD.*

Some portable residential indoor air cleaners generate ozone either intentionally or as a byproduct of operation. Acute and chronic health effects have been linked to ozone inhalation exposure. The U.S. Food and Drug Administration established an ozone emission limit of 50 ppb for medical devices. Underwriters Laboratories (UL) has incorporated the 50 ppb limit into a voluntary standard for emissions from portable household air cleaner products (UL 867). UL 867 allows a maximum steady-state concentration of 50 ppb [0.099 mg/cm^3] in a 30 m^3 test chamber with a net apparent decay rate of 1.33 h^{-1}. CPSC staff used modeling to evaluate the adequacy of UL 867 to maintain ozone at "acceptable" levels (≤ 50 ppb) during normal conditions of air cleaner use in a residential setting. The maximum source strength allowed by UL 867 was estimated to be approximately 4 mg/h. The concentration of ozone in a simulated room or house was then estimated by using one, two, and multiple zone models, a source strength of 4 mg/h, and various input factors (air exchange rate, ambient concentration, deposition velocity, room volume, and surface to volume ratio). Modeled ozone concentrations ranged from 7.73 to 37.4 ppb for six out of seven one-zone scenarios. In the seventh "worst-case," one-zone scenario, reduced infiltration rates, room volume, deposition velocity, ratio of total surface area to envelope surface area, and increased ambient ozone yielded an estimated ozone concentration of 79.8 ppb. In another exercise, probabilistic estimation of ozone concentrations for one-zone and two-zone models ranged from 6.3 to 28 ppb (averages) when using source strengths < 19.8 mg/h. At a source strength of 4 mg/h, approximately 99.9 percent of the estimated cases had ozone concentrations of less than 50 ppb for both the one-zone and two-zone models. Under reasonably foreseeable conditions of use, ozone concentrations of ozone do not exceed 50 ppb when derived from air cleaners that meet the UL 867 voluntary standard. *This work has not been reviewed or approved by, and does not necessarily represent the views of, the Commission.*

PS 1245 SEASONAL CHARACTERIZATION OF HEALTH RISKS DERIVED FROM EXPOSURE TO VOLATILE ORGANIC COMPOUNDS AND BIOAEROSOLS FOR RESIDENTS NEAR A WASTE MANAGEMENT FACILITY.

L. Vilavert, J. L. Domingo, M. Schuhmacher and M. Nadal. *Toxicology, Rovira i Virgili University, Reus, Spain.*

Ecoparc-2 is a large facility which is currently managing an important percentage of the municipal waste organic fraction generated within the metropolitan area of Barcelona (Catalonia, Spain). Because of the concern of the local population and authorities, an environmental surveillance program was recently initiated to control the levels of 19 volatile organic compounds (VOCs) and bioaerosols (bacteria, fungi, and Aspergillus fumigatus) in the surroundings of that facility. Air samples were collected at different distances and wind directions from the Ecoparc-2, in two different campaigns (winter and summer). Mean VOC concentrations were found to be 32.4 and 15.7 μg/m3 ($p>0.05$) in winter and summer, respectively, being BTEX the most predominant VOCs. This is in agreement with previous results obtained from the scientific literature, where an inverse correlation between VOC air levels and temperature was reported. In contrast, higher concentrations of most microbiological pollutants (total bacteria, Gram-negative bacteria, and fungi at 37C) were found in summer, with the only exception of fungi at 25C and Aspergillus fumigatus at 37C. Anyhow, no statistically significant differences were observed between both campaigns, including those related to distances and wind directions. Moreover, "outdoor" levels were various orders of magnitude lower than those previously observed in a recent "indoor" survey (same facility). The current concentrations have been also used to assess exposure to chemical and biological agents for the local population, as well as to evaluate human health risks. It was noted that the levels of VOCs in the vicinity of the Ecoparc-2 should not mean either non-carcinogenic or carcinogenic risks for the subjects living in the neighborhood. These levels were clearly under the threshold values recommended by national and international regulatory organisms.

PS 1246 ENVIRONMENTAL IMPACT OF THE USE OF ALTERNATIVE FUELS IN CEMENT PLANTS: BASELINE STUDY IN TWO FACILITIES.

J. L. Domingo, J. Rovira, M. Mari, M. Schuhmacher and M. Nadal. *Toxicology, Rovira i Virgili University, Reus, Spain.*

In Sant Feliu de Llobregat (SF) and Montcada i Reixac (MR) (Catalonia, Spain), there are two cement plants whose current goal is replacing part of the traditional (fossil) fuel by alternative fuels. We have carried out a six-monthly survey to determine the baseline levels of heavy metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in soil, vegetation, and air samples collected near both facilities. Pollutant concentrations were found to be slightly higher than those reported in the vicinity of other cement plants of Catalonia, being especially close to levels of urban zones. The influence of activity if the SF facility was found to be low in comparison to other potential emission sources in the area, as a decrease of immission concentrations was not registered when the facility ceased temporally its industrial activity. The current results are basic to be used in future studies focused on establishing potential changes in cement plants emissions, and hence to determine the risks for the human health derived from the co-combustion of alternative fuels.

PS 1247 ASBESTOS EXPOSURE ASSOCIATED WITH FIRE SLEEVE MATERIALS.

C. L. Blake[1], S. C. Harbison[2], G. T. Johnson[2] and R. D. Harbison[2]. [1]*Bureau Veritas, Atlanta, GA and* [2]*Environmental and Occupational Health, University of South Florida, Tampa, FL.*

Asbestos containing fire sleeve has been used as a fire protection measure for aircraft fluid hoses. Chrysotile asbestos fabric containing fire sleeves were utilized as a fire protection measure for hose assemblies on aircraft and other high performance engine applications. These asbestos-containing hose assemblies are sometimes removed and installed during engine related work, and are thus manipulated by mechanics. In certain instances chafing of hoses against metal objects has caused erosion of the outer rubber covering, exposing the interior asbestos fabric. The fabrication and subsequent use of fire sleeve protected hose assemblies poses a risk of asbestos fiber exposure to persons involved in the fabrication process, and subsequent aircraft and high performance engine maintenance activities. This investigation was conducted to determine the level of airborne asbestos fiber exposure encountered by mechanics that work with fire sleeve protected hoses. Duplicate testing was performed inside a small-enclosed workroom during the fabrication of hose assemblies. Personal air samples collected during this work showed detectable, but low airborne asbestos fiber exposures. Analysis of personal samples (n=9) using phase contrast microscopy (PCM) indicated task duration airborne fiber concentrations ranging from 0.017 to 0.063 fibers per milliliter (f/ml) for sampling durations of 167 to 198 minutes, and 0.022 to 0.14 f/ml for 30-minute samples. Airborne chrysotile fibers were detected in 4 of these 9 personal samples, in concentrations ranging from 0.014 to 0.025 f/ml. These results indicate that work with asbestos fire sleeve and fire sleeve protected hose assemblies, does not produce harmful levels of asbestos exposure for persons who handle, cut, and fit these asbestos-containing materials.

PS 1248 CHARACTERIZATION OF AN ARS ANIMAL MODEL OF WHOLE-BODY IRRADIATION IN THE MINIPIG.

S. Harbo[2], L. Staska[2], M. Murphy[1], J. Lovaglio[2] and K. D. Thrall[1]. [1]*Battelle, Pacific Northwest Division, Richland, WA and* [2]*Biological Monitoring & Modeling, Battelle Toxicology Northwest, Richland, WA.*

In vivo efficacy studies are necessary to support development of medical countermeasures against radiation injuries. The minipig is an attractive large animal model with a number of physiological characteristics similar to humans. An LD50/30 study was conducted to characterize the dose-response survival and time-course change in hematological and clinical chemistry parameters resulting from whole-body radiation in the Göttingen minipig. Animals were exposed bilaterally to Co-60 at a dose-rate of 0.08 Gy/min for doses of 1.5, 1.7, 1.9, 2.1 and 2.3 Gy. Supportive medical management was not provided, although analgesics were provided. Body weight and temperature were measured daily. Blood was collected from animals daily for hematological analyses and twice a week for clinical chemistry evaluations. Necropsy and histopathology was conducted on all animals at termination. The expected decline in blood components was observed across all exposure groups. Over the 30-day period post-irradiation, all animals at the lowest dose (1.5 Gy) were able to recover, whereas no animal survived whole-body irradiation at the highest level (2.3 Gy). All mortalities occurred between post-irradiation days 17 and 22.

PS 1249 THE ARCTIC DILEMMA: COMMUNITY-BASED OUTREACH RELATED TO PISCIVORY AND MERCURY.

C. Lieske and T. O'Hara. *University of Alaska Fairbanks, Fairbanks, AK.*

The "Arctic Dilemma" describes the conflict between the potential negative health effects of contaminants present and the obvious health promoting nutritional benefits of subsistence foods consumed by Arctic communities. Increased concern for environmental contaminants may threaten the intake of these important traditional foods. To address the Arctic Dilemma, our study balances the analysis of contaminants by also evaluating important nutritional components. We utilize community participatory research methods in order to sample foods (raw and cooked) that are being consumed by the population of concern. Communication of our results to the community is a critical part of the research project. However, this communication is complicated by the presence of varying Federal and international criteria for acceptable human exposure to mercury. For example, in our study 218 raw & 112 processed samples from 177 individual fish (representing approximately 12 species) were analyzed for total mercury (THg) concentration. Wet weight THg ranged from 0.006 ppm in raw capelin to 0.72 ppm in boiled sculpin muscle. All tissues were below the FDA action level for mercury in fish of 1ppm, but some of the samples would fall above the acceptable daily intake limit (ADIL) for mercury depending on the size of the food portion and the weight of the consumer. The proportion of the samples above the ADIL depends on which criteria are utilized. We have simplified our presentation of results though using the recommendations set by Alaska Scientific Advisory Committee for Fish Consumption for a 70 kg person consuming 100g in addition to balancing the risk of fish consumption with the nutritional benefits (such as selenium and fatty acids). Utilizing this strategy facilitates communication, but could lead to misunderstanding or misinterpretation when faced with other consumption recommendations and is contrary to the advice of some national and international guidelines. We face the communication dilemma in the Arctic.

PS 1250 IMPACT OF TOXICITY OF SELECTED PESTICIDES ON SURVIVILITY AND EFFICACY OF AN ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS MINUTUS.*

R. Paul and C. Girish. *Department of Post-Graduate Studies in Zoology, Gulbarga University, Gulbarga, Karnataka, India.* Sponsor: C. Chetty.

Entomopathogenic nematodes (EPNs) including Rhabditida: Steinernematidae and Heterorhabditidae which have insecticidal activity are successfully employed as biocontrol agents against a broad range of insect pests. EPNs are often applied in

conjunction with chemical pesticides under integrated pest management. Heterorhabditis minutus, an EPN has the potential as a biocontrol agent and considered ideal for the local agro climatic conditions. Infective juveniles (IJs) of *H. minutus* isolated from the larvae of tobacco caterpillar *Spodoptera litura* and, the multiplied IJs were harvested in the laboratory. Initially, the IJs were directly exposed to six pesticides (Endosulfon, Acephate, Profenofos, Chlorpyrifos, Methomyl and Cypermethrin), which are used to control the insect pests) by Static Bioassay Tests for 24, 48 and 72 hour exposure in 0.1 to 1.0 percent dilutions of stock solution of each pesticide (field recommended dilution being 0.5 percent). IJs of EPN exhibited 100% mortality after 72 hour exposure to Endosulfon; however, varying degrees of mortality with the other pesticides was observed. The rate of mortality was increased with an increase in the period of exposure. Further, the infectivity of the recovered IJs of the EPN from pesticide exposure was assessed by bioassay method using the later stage larva of shoot and fruit borer Leucinodes orbanalis under laboratory conditions. These recovered IJs after exposure to the five pesticides have successfully infected the pest resulting in the mortality suggesting that the pesticide-exposed and recovered EPN retains the efficacy to infect the pest and cause mortality, however, in varying degrees. The efficacy was found to be highest in those recovered EPNs which were exposed to Cypermethrin followed by those exposed to Methomyl, Chlorpyrifos, Profenofos and Acephate. Results suggest that the EPN is more compatible with the pyrethroid pesticide than with the carbamate or aromatic and heterocyclic organophosphate pesticides, while completely incompatible with organochlorine pesticide.

PS 1251 THE ROLE OF BOUND, SKIN RESIDUES IN DERMAL ABSORPTION OF PESTICIDES.

R. C. Cochran[1] and J. H. Ross[2]. [1]*Risk Assessment, GemQualityRisk, Inc., Sacramento, CA and* [2]*GemQualityRisk, Inc., Carmichael, CA.*

Most human, occupational exposures to pesticides occur through the dermal route. Consequently, an accurate estimate of dermal absorption is critical to assessing systemic exposures to pesticides. Standard FIFRA in vivo dermal absorption studies clearly indicate the percentage of pesticide that has entered a test animal's system from the amount of chemical recovered from the urine, feces, carcass, and cage washes. The question arises, though, as to how the amount of pesticide that is left bound to the skin at the application site should be considered. Estimations of acute risk generally involve toxic endpoints that are expressed in less than 24 hours. Thus, pesticide residues still bound to the skin at the end of 24 hours will not produce an acute, systemic toxic effect. The situation with regards to dermal absorption of repetitive, daily exposure doses is more uncertain. From our examination of twenty guideline dermal exposure studies extending beyond 24 hours, it is apparent that some bound skin residues become systemically absorbed over time. Consequently, there is the possibility that bound skin residues might significantly increase systemic levels of a pesticide in a daily dosing regime. This possibility has led to the practice of USEPA and other regulatory agencies assuming that bound skin residues are part of the systemic exposure through the dermal route. This assumption implies that bound skin residues significantly increase the daily systemic absorbed dose after day 1 in multiple day dermal exposures. Preliminary examination of the data from pesticide dermal exposure studies in which the chemical was not washed off until termination, or the animals were dosed on succeeding days, indicate that the daily systemic absorption of pesticides does not significantly increase after day 1. It is therefore concluded that the bound skin residue should not be used to increase the estimated systemically absorbed dose.

PS 1252 BISPHENOL A (BPA) AND HEXABROMOCYCLODODECANE (HBCD) STEREOISOMERS IN U.S. FOOD.

A. Schecter[1], N. Malik[1], O. Paepke[2] and L. Birnbaum[3]. [1]*Environmental and Occupational Health Sciences, University of Texas School of Public Health, Dallas, TX,* [2]*Eurofins Gfa GmbH Laboratory, Hamburg, Germany and* [3]*National Institute for Environmental Sciences, Research Triangle Park, NC.*

Bisphenol A (BPA) and hexabromocyclododecane (HBCD) are chemicals used in multiple applications. BPA is used in can linings to protect food. HBCD is a flame retardant. These chemicals enter the environment by leaching from consumer products. Exposure occurs primarily through food and dust ingestion. To the best of our knowledge, we report levels of BPA in fresh, canned, and plastic contained U.S. food and HBCD stereoisomers in U.S. food for the first time. Food was collected from Dallas, Texas supermarkets in 2010. 13C-labelled BPA and γ-HBCD were used as internal standards. Control of recovery of internal standards was followed by recalibration within each sequence of analyses. A minimum of one blank was used within each batch. Analyses of BPA and HBCD were by HRGC/LRMS and LC/MS-MS, respectively. BPA was detected in 63 of 105 samples, including human, cat, and dog foods. Levels ranged from 0.23 to 65.0 ng/g wet weight (ww). BPA levels were not necessarily associated with type of food but were higher in canned than fresh food. BPA levels were higher for foods of pH 5 and lower for more acidic and alkaline foods. HBCD was detected in 25 of 36 food samples. Total HBCD levels ranged from 0.023 to 1.363 ng/g ww. Stereoisomer specific analysis detected α-HBCD in 23 samples with levels ranging from 0.006 to 1.307 ng/g ww; β-HBCD in 11 samples with levels from 0.005 to 0.019 ng/g ww; and γ-HBCD in 18 samples with levels from 0.012 to 0.170 ng/g ww. The α-HBCD stereoisomer dominated in these samples as in humans and wildlife despite γ-HBCD being highest in commercial HBCD mixtures. BPA levels did not exceed the reference dose set by the U.S. government. However, health effects of chemical mixtures are unknown. As detectable levels of BPA and HBCD were found in many foods, further research is indicated to determine levels of BPA, HBCD, and other endocrine-active compounds in U.S. food. (This does not reflect NIH policy.)

PS 1253 SECOND-HAND, SMOKE-INDUCED PRO-INFLAMMATORY CYTOKINE PRODUCTION AND OXIDATIVE STRESS IN MICE.

T. Muthumalage[2], K. Hunter[3, 2], D. Redelman[4], K. Pritsos[1] and C. Pritsos[1, 2]. [1]*Nutrition, University of Nevada, Reno, NV,* [2]*Environmental Sciences Graduate Program, University of Nevada, Reno, NV,* [3]*Microbiology and Immunology, University of Nevada, Reno, NV and* [4]*Physiology and Cell Biology, University of Nevada, Reno, NV.*

Exposure to high levels of secondhand smoke (SHS) remains a serious problem for many workers. In this study we looked at SHS stimulated cytokine production and oxidative stress in BALB C and C57BL/6 mice exposed to SHS for 6 hours per day for 3 consecutive days. 24 hours after the final exposure, BAL fluid and lung tissue were collected for analyses. Lung tissue was assayed for SOD and GPOX activity. A time-dependent study (2,6 and 24 hours) established that 24 hours was the best time to collect cytokine data following LPS stimulation of macrophage for the cytokines being tested. BAL was subsequently assayed for GM-CSF, IL-1B, IL-6 and TNF-alpha 24 hours following alveolar macrophage stimulation with LPS or vehicle control. The data show that C57BL/6 and BALB/C mice respond differently to SHS exposure. C57BL/6 unexposed AM show greater cytokine response after LPS stimulation than the BALB/C AM. Following exposure, however, the C57BL/6 SHS exposed AM had a decreased LPS-induced inflammatory response as compared to their unexposed counterparts whereas the BALB/C SHS exposed AM had an equivalent or greater inflammatory response. These results suggest that C57BL/6 mice but not BALB/C may express an endotoxin tolerance similar to that observed in human smokers. . This study was supported in part by a grant from the Flight Attendant Medical Research Institute and the Nevada Agricultural Experiment Station.

PS 1254 COMMONLY USED AIR FILTERS DO NOT SUBSTANTIALLY REDUCE EXPOSURE TO SECONDHAND SMOKE CONSTITUENTS.

C. Pritsos[1, 2] and T. Muthumalage[2]. [1]*Nutrition, University of Nevada, Reno, NV and* [2]*Environmental Science Graduate Program, University of Nevada, Reno, NV.*

Exposure to high levels of environmental tobacco smoke (ETS) remains a serious problem for many workers. Seeking exemptions to smoking bans, some businesses and industries claim that air filtration systems provide adequate protection to workers from ETS, although these claims have little scientific basis. In this study we tested whether air filtration could significantly reduce ETS contaminants. Using a Teague-10 smoking machine to generate SHS we determined carbon monoxide (CO) total suspended particle (TSP), Particulate Matter (PM) 1, 2.5 and 10 levels both before and after air filtration. Air filters commonly used in homes and businesses (MERV ratings of between 4 and 8) were tested. Study results showed that MERV 4 rated filters (commonly used in homes) had no effect on CO levels and little effect on TSP and PM 1, 2.5 and 10 particles (less than 20% reduction following filtration). MERV 6 and 8 rated filters which represent higher efficiency filters used in businesses did not affect CO levels and reduced TSP and PM 1, 2.5 and 10 particulate levels by less than 50% of non-filtered air levels. The significant levels of PM 1 and 2.5 particulates remaining after filtration are of major concern as these are known to be severely damaging to lung tissues. These results demonstrate that air filters commonly used in homes and businesses do not eliminate exposure to ETS chemical constituents. This suggests that common air filtration systems do not eliminate SHS toxicity. This study was supported by a grant from the Flight Attendant Medical Research Institute and by the Nevada Agricultural Experiment Station.

PS 1255 URINARY *T, T* MUCONIC ACID LEVELS ARE MODULATED BY *EPXH1H139R* AND *NQO1*2* POLYMORPHISMS IN CHILDREN.

E. Jiménez-Mendoza[1, 2], M. Sánchez-Guerra[1], N. A. Pelallo-Martínez[3], F. Díaz-Barriga[3], L. Carrizales-Yánez[3] and B. Quintanilla-Vega[1]. *[1]Toxicology Department, CINVESTAV-IPN, Mexico City, D.F., Mexico, [2]FES-Iztacala-UNAM, Mexico City, Mexico and [3]Faculty of Medicine, UASLP, San Luis Potosi, Mexico.*

The main adverse effect of chronic exposure to benzene is leukemia and other cancers, representing a public health problem, especially for children, who are one of the most vulnerable populations. After being metabolized, benzene is eliminated in urine as *trans,trans*-muconic acid (*t,t*-MA), which is a sensitive indicator of low benzene exposures. Some enzymes involved in benzene metabolism such as CYP2E1, EPHX1 (activation), and NQO1 and GST (deactivation) have polymorphisms that may lead to greater susceptibility in exposed subjects. Coatzacoalcos County, in Veracruz, Mexico (in the Gulf of Mexico), is characterized as an area of great industrial activity mainly petrochemical. We conducted a cross-sectional study in children (6 to 10 years old, n=85) from three elementary schools, located near the major industrial complexes. Urine and blood samples were collected, and urine *t,t*-MA levels were determined by HPLC and genetic polymorphisms *CYP2E1 PstI/RsaI*, *GSTM1*0* and *GSTT1*0* by conventional PCR and *EPHX1T113H*, *EPHX1H139R* and *NQO1*2* by real-time PCR. Thirty percent of children had higher urinary levels of *t,t*-MA than the biological exposure index in the workplace (500 μg *t,t*-MA/g creatinine). Results about the gene-environment associations showed that *EPHX1H139R* heterozygous children had significant lower *t,t*-MA levels (-36%, p=0.002), a polymorphism associated with an increased activity (8% allele frequency) as well as *NQO1*2* homozygous children (-51%, p=0.011), a polymorphism associated with a decreased activity; these compared to wild-type individuals. There were no homozygous subjects for *CYP2E1 PstI/RsaI* polymorphism. Our results suggest that *EPHX1H139R* and *NQO1*2* polymorphisms modulate urinary *t,t*-MA levels in children environmentally exposed to benzene, which may contribute to individual metabolic differences and therefore, higher susceptibility to develop toxic effects. (Supported by CONACyT Grant-87234).

PS 1256 BLOOD MERCURY CONCENTRATION AND RELATED FACTORS IN AN URBAN COASTAL AREA IN KOREA.

Y. S. Hong[1], D. S. Kim[2], E. M. Jo[1], B. G. Kim[1], Y. M. Kim[1], C. H. You[1], S. D. Yu[2] and J. D. Park[3]. *[1]Preventive Medicine, Dong-A University, Busan, Republic of Korea, [2]Environmental Epidemiology, National Institute of Environmental Research, Seoul, Republic of Korea and [3]Preventive Medicine, Chung-Ang University, Seoul, Republic of Korea.*

Objectives: This study was carried out for the purpose of evaluating the blood mercury concentration of the residents of Busan, Korea, as well as the relationship between the mercury concentration and the pattern of fish consumption along with other epidemiological factors. Methods: Two hundred ninety-three subjects (147 men and 146 women), who were aged 40 years or more, were recruited into this study between June and October 2009. The mean age of the subjects was 54.3 years (with a range of 40-70 years). Mercury concentrations in blood samples were measured using a gold-amalgam collection method. Results: The geometric mean concentration of mercury in the total subjects was 8.63 μg/L [range: 1.48~45.71 μg/L]. The blood mercury concentration of the men (9.55 μg/L) was significantly higher than that of the women (7.76 μg/L). The blood mercury concentration of those who eat fish more than 4 times per week was higher than others, and was statistically significant (male p=0.0019, female p=0.0002). According to the multiple analysis, the blood mercury concentration was significantly affected by the consumed fish but other epidemiological factors were not related. Conclusions: It was found that the subjects who have consumed a large amount of fish may have high blood mercury concentration. It appears that fish consumption can influence blood mercury concentration. Therefore, guidelines for fish consumption that will decrease blood mercury concentration might be necessary in Korea.

PS 1257 HIGH PERFORMANCE METABOLIC PROFILING (HPMP) FOR ENVIRONMENTAL TOXICOLOGY.

Y. H. Park. *Medicine, Emory University, Atlanta, GA.* Sponsor: D. Jones.

Over 60,000 chemicals are registered for commercial use, and each of these can be biologically or environmentally altered to give additional xenobiotics. Yet modern surveillance tools largely rely upon approaches of analytical chemistry in which chemicals are analyzed one at a time against authentic standards. In the current study, we used liquid chromatography coupled to high resolution mass spectrometry and algorithms for peak extraction to detect and provide relative quantification of about 4000 chemicals, both known and unknown, in small volumes of plasma from humans and 6 other mammalian species. Searches of metabolic and chemical databases for the high resolution m/z showed matches to endogenous metabolites, dietary components and environmental chemicals such as pirimicarb (insecticide), triphenylphosphate (plasticizer and fire retardant) and pencycuron (fungicide). However, greater than half of the detected m/z did not match known chemicals, indicating that a substantial range of unidentified chemicals are also commonly present in human and other plasma. Controlled experimental studies show that effects of exposures on patterns of endogenous metabolites can readily be detected. Routine application of this method would provide a capability to obtain a new level of understanding of geographic and life-cycle differences in exposures and provide the basis to link specific exposures to underlying metabolic responses.

PS 1258 EVALUATING CHEMICAL EXPOSURES USING HEMOGLOBIN ADDUCTS FOR ACRYLAMIDE AND 1, 3-BUTADIENE.

J. M. Shimek[1], B. McCarthy[2], S. Erdal[1] and F. Davis[2]. *[1]Environmental and Occupational Health Sciences, University of Illinois at Chicago, Chicago, IL and [2]Epidemiology and Biostatistics, University of Illinois at Chicago, Chicago, IL.*

Hemoglobin (Hb) adducts reflect exposure to specific chemicals occurring in the past four months. Improvements in chemical-specific analytical methods permit the measurement of low levels of Hb adducts and the results are an indicator of exposure from all potential sources. Interpretation of results is difficult because of the many sources of chemical exposure and variability in humans. In this study, exposure to acrylamide and 1,3-butadiene was examined to assess the influence of occupation, demographics, tobacco use and diet on Hb adduct formation. Seventy-six participants were recruited from an existing control population of a glioma case-control study. Participants had completed an extensive questionnaire and blood samples were collected at the time of initial enrollment or on a return visit. Analysis for the *pyr*-Val adduct of 1,3-butadiene was completed at UNC, Chapel Hill and for the acrylamide adducts at the CDC Environmental Sciences lab. Current occupation was compared with the 1981-83 National Occupational Exposure Survey to determine the potential for exposure. Questionnaire data was compared to the resulting levels of pyr-Val adducts for 1,3-butadiene and acrylamide and glycidamide adducts for acrylamide. Potential occupational exposures did not show a difference between the exposed and unexposed subjects. When comparing geometric means of subgroups, statistically significant differences (α=0.05) were found for the pyr-Val adduct for state of residence and second hand smoke exposure; for acrylamide adducts for current and ever smokers; and for glycidamide adducts for increased consumption of wheat, corn or millet products. Detection of the *pyr*-Val adduct in humans has not previously been reported and is significant because the epoxide that forms this adduct is the most carcinogenic of the three 1,3-butadiene adducts. The acrylamide adduct results are significant for smoking and diet history. Continuing research needs to be done in this area.

PS 1259 A FISHING LINE GENERATOR TO DELIVER WTC DUST PARTICLES FOR INHALATION EXPOSURE.

J. M. Vaughan, B. J. Garrett, M. D. Cohen and L. Chen. *Environmental Health Science, New York University, Tuxedo, NY.*

First Responders exposed to caustic dust & toxic pollutants following the 2001 World Trade Center (WTC) terrorist attacks suffered irrefutable damage to their respiratory health. Existing studies that have been performed thus far have primarily used only one size fraction (< 2.5μ diameter) of WTC dusts to evaluate health effects; however, this was not truly representative of exposures Responders underwent. No studies have taken into account larger particle sizes normally deemed not biologically relevant (> 10μ diameter) despite the fact the atmospheres contained primarily (>95%) coarse/supercoarse particles and a majority of Responders underwent heavy mouth breathing during their labors at Ground Zero. Thus, analysis of health effects arising from exposures to the larger more alkaline WTC particles is essential. For this, a Fishing line system for delivering large particles was constructed to create relevant exposure scenarios for rats that will correspond to both dust dosages/size distributions the First Responders faced. Further, this system was integrated into an intratracheal inhalation (ITIH) system to provide a relevant inhalation approach (mimics Responders mouth breathing scenarios). Using concentrations (33, 66, 99 mgdust/m3) designed to deliver rat equivalents that correspond to low-high estimated dust levels [250-750 mg/m3] likely encountered by Responders on 9/12-13, particle delivery was measured gravimetrically and size distribution via an elutriator. Male F344 rats (n=8/group) were then exposed to WTC dusts/filtered air via ITIH exposure for 2hr/d for 3 consec days. Lung tissues were collected at several timepoints after the final dust exposure for lung burden analysis/lavage to measure inflammatory parameters. Inflammation was observed at several timepoints post final dust exposure. Via these novel systems, we can conduct

relevant in vivo exposures to determine if WTC dusts caused adverse outcomes in respiratory function & provide a tool hereafter for the generation of (and host exposure to) larger particle fractions.

PS 1260 SENSING OF THE CYANIDE METABOLITE 2, 4 AMINOTHIAZOLINE CARBOXYLIC ACID WITH SURFACE ENHANCED RAMAN SPECTROSCOPY – STEPS TOWARD A PORTABLE SENSOR.

D. E. Thompson[1], A. Parbatani[1], T. Tessema[2], A. Meyers[1] and I. Petrikovics[1]. *[1]Chemistry, Sam Houston State University, Huntsville, TX and [2]Chemistry, Villanova University, Villanova, PA.*

Portable field detectors of cyanide intoxication would be useful in triaging victims of fires on location. We have assembled a surface enhanced Raman spectrometer (SERS) that uses expanded beam sensing to monitor the presence of the cyanide metabolite 2,4 aminothiazoline carboxylic acid (ATCA). This is a spectrometer that is built on a laser table, but which has the potential to be miniaturized for portability. The film-over-nanosphere and nanoprism array sensing surfaces on which ATCA is detected were produced using evaporation induced self-assembly of polystyrene spheres on glass followed by gold deposition. The detailed design of the spectrometer is explained. Specific attention is given to the elements of the spectrometer design that are targeted at achieving a detection limit for ATCA in the biologically relevant nM range. Scanning electron and optical microscope images characterize the SERS sensing surfaces on which ATCA is detected. Preliminary SERS spectra of aqueous ATCA solutions, collected using a 785nm pump laser, demonstrate promising proof-of-principle for the method.

PS 1261 BTEX SERUM LEVELS IN THE GENERAL U.S. POPULATION: AN ANALYSIS OF THE 2003-2004 NHANES DATASET.

J. D. Urban, L. Fitzgerald, B. Burkhalter, D. Staskal-Wikoff, M. Harris and L. Haws. *ToxStrategies, Inc., Austin, TX.*

Benzene, toluene, ethylbenzene and xylenes (BTEX) are volatile organics compounds that are commonly found in many solvents and petroleum products. Although BTEX compounds are rapidly metabolized and eliminated in the human body, persistent exposure can result in a measurable internal dose. The National Health and Nutrition Evaluation Survey (NHANES) gathers data on a host of chemicals found in the general public and, as such, are useful for interpreting data gathered during exposure investigations designed to assess whether particular individuals are being exposed to chemicals in their environment. The objective of this investigation was to derive serum reference values for the national population using BTEX serum concentration data (ng/ml) from the 2003-2004 NHANES dataset. Weighted summary statistics were calculated for BTEX serum levels using NHANES subset-specific 2-year weights. Data were evaluated by several demographic variables including gender, age, and ethnicity, as well as by smoking status based on two biomarkers for smoking [2,5-dimethylfuran (DMF) and cotinine]. Consistent with prior analyses of earlier NHANES datasets, data distributions for all compounds were right-skewed. *m/p*-Xylene (median: 0.13, range: 0.02-5.6) and toluene (0.1, 0.02-9.0) had the highest serum levels, followed by benzene (0.03, 0.02-1.4), *o*-xylene (0.03, 0.02-1.5), and ethylbenzene (0.03, 0.02-1.6). No significant trends were noted when serum concentrations were evaluated by demographic variables. However, smokers had elevated mean and median serum levels for all compounds except *o*-xylene regardless of the smoking biomarker. While historically cotinine has been the standard smoking biomarker, DMF is now believed to be a better measure of smoking because it is a direct by-product of tobacco smoke. We found serum BTEX levels in smokers were higher when using DMF as the biomarker for smoking activity regardless of statistic (mean, median, 95th percentile). In conclusion, these summary statistics are useful reference values for investigators assessing exposures to BTEX compounds.

PS 1262 PROFILING THE INTERNAL DOSE OF REACTIVE AGENTS AND THEIR METABOLITES USING N-TERMINAL VALINE ADDUCTS.

S. Goel, N. I. Georgieva, M. Thompson and G. Boysen. *Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR.*

Humans are constantly exposed to various mixtures, such as tobacco smoke, auto exhaust, and other environmental and occupational pollutants, containing several thousand compounds, including many known carcinogens. DNA and protein adducts are well established biomarkers for the internal effective dose and are an integral part of science-based risk assessment. Technical limitations, however, have prevented comprehensive assessment of a broad spectrum of different adducts simultaneously. Consequently, most studies have focused on determination of abundant individual or a selected few adducts. These studies have produced valuable insights into metabolism of individual carcinogens, but they are insufficient in providing accurate data needed for assessment of the risk posed by exposure to mixtures. To overcome this limitation we present herein a sensitive and specific method for quantitative profiling of a broad spectrum of reactive compounds or their metabolites using hemoglobin adducts as surrogate biomarkers. The reported method is based on our previously established immunoaffinity liquid chromatography–tandem mass spectrometry (LC-MS/MS) method. To enable simultaneous quantitation of a broad spectrum of N-terminal valine adducts the immunoaffinity chromatography was modified and adduct profiles were obtained by full scan mass spectrometry. The method has been validated with in vitro treated human and mouse globin. Subsequently the method was applied to generate exposure profiles from various rodent inhalation studies. This novel concept enables determination of the internal doses of several carcinogens simultaneously to better understand the behavior of individual compounds in mixtures, identify novel, uncharacterized alkylating agents, elucidate potential compound–mixture interactions, and generate more comprehensive exposure data needed for accurate risk assessment of exposure to mixtures.

PS 1263 EVALUATION OF AIRBORNE TOXICANT CONCENTRATIONS FROM THE DEEPWATER HORIZON OIL SPILL.

J. Keenan[1], H. Avens[2], K. Unice[3] and D. Paustenbach[1]. *[1]ChemRisk, San Francisco, CA, [2]ChemRisk, Boulder, CO and [3]ChemRisk, Pittsburgh, PA.*

The Deepwater Horizon oil spill in the Gulf of Mexico has surpassed the Exxon-Valdez oil spill as being the largest in U.S. history. Oil recovery and remediation workers are potentially exposed to volatile toxicants while on boats on or near oil spills. These volatile toxicants include, but are not limited to, benzene, ethyl benzene, toluene, and xylene. British Petroleum (BP) has made tens of thousands of IH samples available for review. Each measurement had an associated job/task/location descriptor assigned by BP which were not consistent in their terminology and were not readily useful for doing an analysis. In order to analyze this large dataset and to determine if specific jobs, tasks, or locations were associated with higher chemical concentrations, the available BP data was grouped into 35 new task categories that described approximately 25% of the data. The task categories include actions such as deploying boom, applying dispersants, taking water samples, and performing skimming operations. Task categories were grouped into a lower, middle, and upper exposure level for each chemical. The vast majority of the data were in the middle group which comprised the task categories whose geometric means were within half to two-fold the geometric mean for all the data. The analysis revealed that measurements did not vary significantly from task to task and most measurements were low. The geometric means for the middle level exposure group were 7.7 (+/- 1.6), 26 (+/- 2), 26 (+/- 2), and 63 (+/- 2) ppb for benzene, ethylbenzene, toluene, and xylene, respectively. These data will be used to evaluate a simple model for characterizing airborne toxicant concentrations resulting from a continuous deepwater release of oil.

PS 1264 IDENTIFYING AND COMMUNICATING EXPOSURES AND HEALTH RISKS FROM MICROCONSTITUENTS AT BIOSOLIDS LAND APPLICATION SITES.

V. B. Mylavarapu[1] and P. V. Cline[2]. *[1]EE&S, CH2M Hill, Orlando, FL and [2]EE&S, CH2M Hill, Gainesville, FL.*

Land application of biosolids is a technology considered by EPA as the option that promotes beneficial reuse while causing least harm to the environment. Public health concerns are raised about exposures to residual microconstituents in soil, groundwater and surface water. This case study addressed concerns of nearby residents where biosolids were land farmed for over 25 years. The biosolids were from domestic wastewater, hospitals and a large university. The residents attributed occurrence of thyroid cancer and reproductive abnormalities of farm animals to exposures to dust and groundwater contaminated with pharmaceuticals/endocrine disrupting chemicals land applied biosolids. In response, a sampling strategy was developed to identify and analyze chemical residues in biosolids. A commercial laboratory with validated protocols was selected for analyzing hormones, pharmaceuticals, and industrial endocrine disruptors. The results were compared against concentrations at other sites and toxicity data. Majority of the chemicals analyzed did not have toxicity factors from EPA or other published sources. For example only 5 out of 35 chemicals selected for analysis had peer reviewed toxicity factors. A subset of the 35 chemicals was selected to allow sample numbers within budget, accom-

modating a reliable data evaluation given the high false positives potential at part per trillion levels. None of the analytes were detected in soil-leachate and groundwater samples. Fluoxetine was detected in the leachate of biosolids and the implications were evaluated. While this study was based on agreed upon protocol, challenge is to prove "safe" conditions. Public continually request additional analysis as new analytical methods evolve. Therefore, instead of individual chemical analysis and risk estimations, biomarker assays that measure response to chemical groups could serve as alternative analytical tools at biosolids facilities. This project also addressed effective public communication challenges and the perceptions of exposures to large mixture of microconstituents.

PS 1265 SITE EVALUATION OF BIOACCESSIBILITY OF DIOXINS/FURANS FROM SOIL.

Y. W. Lowney[1], G. Brorby[2] and R. Kalmes[2]. [1]*Exponent, Boulder, CO and* [2]*Exponent, Oakland, CA.*

A bioaccessibility extraction test for dioxins/furans in soil was implemented for soils from a former magnesium plant in Nevada. Fingerprint analyses indicated that the majority of the congeners were furans, particularly octafurans, whereas most of the published studies on bioaccessibility were conducted on 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). Therefore, the objective of the study was to provide site-specific information to understand the relative oral bioavailability of dioxins/furans from site soils (on a toxicity equivalent [TEQ] basis). Ten surface soil samples were collected from areas of the site where data indicated that dioxin/furan TEQ concentrations generally ranged between 1000 and 3000 ppt and that included a representative range of organic carbon content, the latter having been shown to affect bioaccessibility. The soil samples were allowed to air dry, sieved to the <250 um particle size fraction, and extracted using a sequential extraction procedure that simulates a stomach phase, followed by a small-intestine phase. Aliquots of the original soil samples and the extraction fluid were analyzed according to EPA Method 1613. The bioaccessibility of the dioxins/furans was calculated, on a congener-specific basis, by comparing the mass of each congener in the extraction fluid to the mass of each congener present in the soil that was subjected to extraction. Percent bioaccessibility for total TEQ ranged from 10% to 31%, with an overall mean of 20% (excluding one sample result of 5%, which appeared anomalously low). On a congener-specific basis, bioaccessibility values ranged from 6% to 43%, with no clear pattern in extraction efficiency across the different soils. These values are in the same range as those reported for relative oral bioavailability from animal studies, particularly those based on swine, which is considered to be the preferred test species over rodents because of gastrointestinal physiology that is more similar to humans. The results of this evaluation of bioaccessibility were used to support site-specific soil remediation target levels and remedial decision.

PS 1266 ASSESSMENT OF BENZENE SHOWER EXPOSURES - IMPLICATIONS FOR SOIL AND WATER QUALITY GUIDELINE DEVELOPMENT.

A. L. Knafla[1], S. Hays[2], J. Campbell[1] and M. Morden[3]. [1]*Equilibrium Environmental Inc., Calgary, AB, Canada,* [2]*Summit Toxicology, Lyons, CO and* [3]*Suncor Energy Inc., Calgary, AB, Canada.*

Benzene soil quality guidelines in Canada are based on protecting supplies of potable water such as groundwater. Historically, benzene exposure through water has been assessed via oral consumption. More recently, regulatory guidelines have incorporated dermal and inhalation exposure due to showering and bathing activities. A relatively simplistic and conservative screening model, in terms of exposure and dose estimation, was implemented in Canada as part of a draft water quality guideline update to incorporate exposures via these routes for summation with oral exposure. Results indicated that inhalation and dermal exposures for benzene can be significant, and should be incorporated into water guideline development.
To provide a more detailed evaluation of exposures to benzene and potential doses to target tissue, a mass balance exposure module integrated with a physiologically-based pharmacokinetic (PBPK) model was developed for estimating time-dependent cumulative multi-pathway exposure and dose metrics associated with drinking water and showering/bathing activities. The exposure model was calibrated against published data on shower and building air concentrations. Time curves for benzene concentrations in venous blood were generated in addition to estimates of total daily benzene metabolism to provide dose metrics for assessing the potential relative contribution of different exposure pathways towards target tissue dosing. For context, the resulting dose metrics were compared with metrics associated with an inhalation risk specific dose (RsD) used by regulatory agencies. Oral exposure resulted in the greatest average venous benzene concentration, followed by inhalation and dermal exposure in descending order. These differences were augmented when compared on a total daily benzene metabolism basis. A benzene water concentration of 0.005 mg/L produced benzene blood concentrations and total metabolite formation rates that were lower than those produced by inhalation exposure at the RsD.

PS 1267 ERIONITE MINERAL FIBERS IN ROAD GRAVEL: A POTENTIAL OCCUPATIONAL HAZARD IN THE WESTERN UNITED STATES.

C. R. Partridge[1], P. H. Ryan[2], S. Adjei[3], J. E. Lockey[3] and S. Griffin[1]. [1]*U.S. EPA Region 8, Denver, Co.,* [2]*Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH and* [3]*Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, OH.*

Environmental exposure to erionite, a carcinogenic zeolite mineral fiber, has been associated with localized and diffuse pleural thickening, interstitial fibrosis, and mesothelioma in Turkey. Heretofore, environmental and occupational exposure to erionite in North America was thought to be uncommon with unknown health consequences. However, gravel excavated in areas of Western North Dakota and used to surface local roads has now been shown to contain erionite. This study was conducted to identify early chest radiographic changes among North Dakota residents with the highest potential exposure to dust from road gravel containing erionite. Residents of Western North Dakota with the highest potential exposure to road gravel containing erionite were eligible for enrollment in the study. Participants completed a study questionnaire, chest X-ray, and high-resolution computed tomography (HRCT) scan to assess the prevalence of interstitial and pleural changes consistent with fibrous mineral exposure. Due to the low population density and rural nature of the study area, a total of 34 individuals met the criteria of the study and were enrolled. The most frequently reported pathway of potential erionite exposure from the study participants was employment in gravel pits and/or road maintenance occupations. Two participants with no reported historical asbestos exposure demonstrated unilateral and bilateral lower lobe interstitial changes and bilateral localized pleural changes with calcification on HRCT. A third participant demonstrated extensive bilateral localized pleural changes with calcification both on chest X-ray and HRCT and no interstitial changes. All three reported extensive work experience in gravel quarries and/or road maintenance. These data indicate that within the United States, erionite exposure may represent a potential occupational health hazard.
(These views are the authors and do not represent USEPA policy)

PS 1268 RISK CHARACTERIZATION OF THE BROMINATED FLAME RETARDANT DECABROMODIPHENYL ETHANE IN INDOOR DUST.

D. G. Dodge[1], M. C. Pollock[2], S. N. Sax[2], C. Petito Boyce[3] and J. E. Goodman[2]. [1]*Gradient, Bend, OR,* [2]*Gradient, Cambridge, MA and* [3]*Gradient, Seattle, WA.*

Brominated flame retardants (BFRs) are added to combustible materials to delay or prevent the ignition or spread of a fire, thereby saving lives and property. Indoor dust is thought to be a major source of human exposure to BFRs as a result of their release from commercial products. Decabromodiphenyl ethane (DeBDethane; CAS # 84852-53-9) is an emerging general purpose BFR. We performed a human health risk characterization for incidental ingestion of and dermal contact with DeBDethane in residential indoor dust. In a comprehensive review and analysis of studies with individual sample results, we found that the geometric mean (and 95th percentile) bulk dust concentrations in residences was 29 (918) ng/g, with similar values in workplaces, cars, and a hotel. Based on a probabilistic exposure assessment framework, we estimated average (95th percentile) daily intakes for DeBDethane of 0.99 (3.1) ng/kg-d for children and 0.11 (0.37) ng/kg-d for adults. A NOAEL value of 1,000 mg/kg-d for DeBDethane was identified from repeated dose toxicity studies. Based on these data, we calculated margins of exposure (MOEs) for DeBDethane of 1 x 10^9 for children and 9 x 10^9 for adults based on average daily intake and 3 x 10^8 for children and 3 x 10^9 for adults based on 95th percentile intake. These results demonstrate large margins of safety for exposures to DeBDethane in indoor dust, even when assuming ongoing exposures in a residential setting.

PS 1269 LIPOSOMAL-NAC PROTECTS AGAINST ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

K. Pucaj[1], C. Buonocoro[2], M. Alipour[2], A. Omri[2], M. Zsabo[2] and Z. Suntres[2]. [1]*Nucro-Technics, Scarborough, ON, Canada and* [2]*Medical Sciences Division, Northern Ontario School of Medicine, Thunder Bay, ON, Canada.* Sponsor: G. Shopp.

Acetaminophen (APAP) is an antipyretic analgesic drug that causes depletion of glutathione and hepatotoxicity when taken in overdose. High doses are associated with production of ROS during formation of N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 which also participate in APAP-induced hepatotoxicity. NAC, best known for its ability to counter acetaminophen toxicity, is a safe, well-tolerated antidote for cysteine/GSH deficiency. The objective of this study was

to determine whether or not the antioxidant N-acetylcysteine delivered in liposomes (phospholipid vesicles used as drug delivery systems) is more effective than the conventional N-acetylcysteine in protecting against acute APAP- induced injuries. Male Sprague-Dawley rats were challenged with an intragastric dose of APAP (850 mg/kg b.wt); 4 h later, animals were administered saline, N-acetycysteine, or liposomal-N-acetylcysteine and sacrificed 24 h post-APAP treatment. Acetaminophen administration resulted in hepatic injury as evidenced by increases in plasma AST and ALT enzymes, increases in tissue levels of lipid peroxidation, myeloperoxidase activity, and nitrotyrosine. Treatment of animals with L-NAC was significantly more effective than treatment with the free drug in reducing APAP-induced hepatotoxicity as indicated by the biochemical markers used in this study. Histological evaluation showed that APAP caused periacinar hepatocellular apoptosis and/or necrosis of hepatocytes around the terminal hepatic venules which was reduced by NAC treatment, the degree of reduction in necrosis being greater for L-NAC. These data suggest that NAC when delivered as a liposomal formulation further improves its effectiveness in counteracting APAP-induced hepatotoxicity. This work was funded by the Northern Ontario School of Medicine.

PS 1270 PARAOXONASE 2(PON 2) IN THE CENTRAL NERVOUS SYSTEM: A NEUROPROTECTIVE ROLE?

L. G. Costa[1, 3], G. Giordano[1], T. B. Cole[2] and C. E. Furlong[2]. [1]*Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA,* [2]*Division of Medical Genetics and Department of Genome Sciences, University of Washington, Seattle, WA and* [3]*Department of Human Anatomy, Pharmacology and Forensic Science, University of Parma Medical School, Parma, Parma, Italy.*

Paraoxonase 2 (PON 2) is a member of the paraoxonase family of proteins which also includes PON1 and PON3. PON2 is widely distributed, with substantial expression in liver, intestine, testis, placenta, heart and macrophages. Several lines of evidence indicate that PON2 acts as an intracellular antioxidant. Aim of the present study was to characterize the expression of PON2 in mouse central nervous system and to assess its antioxidant properties in this tissue. We first confirmed by Western blotting that PON2 levels are highest in lung, intestine, heart and liver, and lower in brain; we also found that in all tissues PON2 expression was higher in female than in male mice. PON2 knockout [PON2-/-] mice did not express any PON2, as expected. In brain, the highest levels of PON2 were found in the substantia nigra, the nucleus accumbens and the striatum, with lower levels in the cerebral cortex, hippocampus and cerebellum. A similar regional distribution of PON2 activity (measured by dihydrocoumarin hydrolysis) was also found. PON3 was not detected in any brain area, while PON1 was expressed at very low levels (20 to 40-fold less than PON2), and did not show any regional difference. PON2 levels were higher in astrocytes than in neurons isolated from all brain regions, and were highest in cells of both types isolated from the striatum. Mouse brain PON2 protein levels decreased from postnatal day 1 to 60. The toxicity in striatal and cerebellar cells of agents known to cause oxidative stress (DMNQ, H2O2, MPP+) was 4 to 11-fold higher in neurons and astrocytes from PON2-/- mice than in the same cells from wild-type mice. These results indicate that PON2 is expressed in brain, and that higher levels are found in dopaminergic regions such as the striatum, suggesting that this enzyme may provide protection against oxidative stress-mediated neurotoxicity (Supported in part by ES04696).

PS 1271 COMPARATIVE EVALUATION OF TAURINE AND THIOTURINE FOR THEIR EFFECTS ON ETHANOL-INDUCED OXIDATIVE STRESS IN THE RAT.

M. C. Parikh and C. A. Lau-Cam. *Pharmaceutical Sciences, St. John's University, Jamaica, NY.*

The present study has evaluated taurine (2-aminoethanesulfonic acid, TAU) and thiotaurine (2-aminoethanethiosulfonic acid, TTAU), two sulfur-containing amino acids, for their effects in attenuating ethanol-induced oxidative stress. To this end, fasted male Sprague-Dawley rats (225-250 g) were used throughout. Animals in the treatment groups received either TAU or TTAU intraperitoneally at a 2.4 mM/kg dose, given 30 min before an oral (4 g/kg) dose of ethanol. Animals in the control group received only physiological saline and those in the no treatment group only ethanol. At 1 hr post ethanol, blood was collected by cardiac puncture into heparinized tubes and the respective livers removed immediately. The blood samples were processed for their plasma fractions, and the livers were made into a homogenate with phosphate buffered saline (PBS) pH 7.4 (1:30 ratio, w/v). These samples were analyzed for the levels of malondialdehyde (MDA), reduced (GSH) and oxidized (GSSG) glutathione, and the activities of catalase, glutathione peroxidase and superoxide dismutase. In comparison to control values, ethanol raised the MDA and GSSG values while lowering those of GSH and antioxidant enzymes both in the plasma and liver. A pretreatment with either TAU or TTAU led to abolishment of MDA formation, to normalization of the GSH stores, and to significant attenuation of the changes in GSSG, GSH/GSSG ratio and activities of antioxidant enzymes induced by ethanol. In general, TTAU was found to be somewhat more protective than TAU against the actions of ethanol. In addition, parallel experiments with other doses of TAU (1.2 and 4.8 mM/kg) indicated that the protective actions of this test compound are dose related and only significant above 1.2 mM/kg.

PS 1272 THE EFFECT OF ESTROGEN ON MACROPHAGES RESPONSE TO OXIDATIVE STRESS.

T. Entezarizaher[1], M. Javdan[2] and Z. Zakeri[3]. [1]*LaGuardia Community College, Long Island City, NY,* [2]*The Feinstein Institute for Medical Research, Manhasset, NY and* [3]*Queens College and Graduate Center of City University of New York, Flushing, NY.*

Macrophages play essential roles in immunity and the maintenance of homeostasis. Several studies have shown that males and females respond differently to traumatic injury, sepsis, chronic pain, fibromyalgia, rheumatoid arthritis and neurodegenerative disease, in which sex hormones are most likely responsible for the differential outcomes. Infectious diseases such as sepsis are a dysregulation of innate immune response to an offending pathogen. Macrophages by recognizing pathogens, phagocytosing them, and secreting inflammatory mediators are the initiator of the innate immune response. The function of macrophages can be influenced both directly and indirectly by the cellular redox state. Macrophage malfunctions have been previously reported under oxidative stress such as hyperoxia and hypoxia; How ever role sex hormones on macrophage function and involved mechanisms remain unclear. In this study we explore the effect of estrogen and testosterone on cell viability damage and phagocytosis impairment induced by H2O2 in Raw 264.7 Cells. Cell viability and phagosytosis function evaluated by MTT and phagocytosis assay. Our data suggest that estrogen has significant protective effect on cell viability and phagocytotic function of macrophages compared to non-treated control cells and the cells that treated with testosterone. Thus this study opened a new window to propose a novel and efficient treatment for sepsis and other gender-related inflammatory diseases.

PS 1273 DOSE-RESPONSE ANALYSIS OF POTASSIUM BROMATE-INDUCED DNA DAMAGE IS CONSISTENT WITH LOW-DOSE LINEAR, NON-THRESHOLD PROCESSES.

M. A. Spassova[1], D. J. Miller[1], J. Caldwell[1], S. V. Vulimiri[1], D. A. Eastmond[2], C. Chen[1] and P. D. White[1]. [1]*NCEA, U.S. EPA, Washington, DC and* [2]*Cell Biology & Neuroscience, University of California Riverside, Riverside, CA.*

Reactive oxygen species (ROS) are generated under physiological and pathological conditions. ROS can arise as a result of enzymatic and nonenzymatic reactions involving ozone, various carcinogens and environmental pollutants. It has been postulated that ROS have both direct and indirect roles in mutagenesis and carcinogenesis. However, the specific mechanisms involved in these processes and the resulting dose-response behaviors are the subject of ongoing debate. In this study, we have focused on investigating the dose-response behavior of DNA damage induced by potassium bromate (KBrO3). We selected KBrO3 as an example of a ROS-generating compound, because substantial amount of evidence suggests that KBrO3 and/or its metabolites directly interact with DNA residues to produce oxidative damage. We evaluated the dose-response of KBrO3 induced DNA damage at the molecular, cellular, and organism level. It has been established previously that at molecular (cell-free) level, when bacteriophage PM2 DNA is used, DNA damage increases linearly with concentration of KBrO3. We used studies that assess KBrO3 -induced DNA damage in human lymphoblastoid and mouse lymphoma cells to evaluate the dose-response at the cellular level. Linear models and models with plateau effects (here bent-hyperbola model) were used to describe the dose-response dependence. Studies on micronucleus (MN) induction by KBrO3 in mouse blood cells in-vivo were used to assess the KBrO3 effect at the organism level. These data were modeled with an exponential model that revealed low-dose linear behavior. Our analysis finds that the observed data on DNA damage induced by KBrO3 is consistent with low-dose linear behavior at the molecular, cellular and organism level, with no indication of a threshold. Disclaimers: The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

PS 1274 ALL-TRANS-RETINOIC ACID PROTECTS AGAINST ROS INDUCED KIDNEY INJURY.

S. S. Lau, J. L. Lord-Garcia, L. W. Lai, K. Y. Yong, Y. H. Lien and T. J. Monks. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Quinones are electrophilic and capable of forming covalent adducts with proteins and of redox cycling with the generation of reactive oxygen species (ROS). Within renal proximal tubular epithelial cells, multi-substituted GSH conjugates of 1,4-

benzoquinone generate ROS and arylate proteins, causing tubular necrosis and the eventual formation of renal carcinomas. Retinoid signaling is engaged during 11-deoxy-16,16-dimethyl PGE2 (DDM-PGE2) mediated cytoprotection against ROS induced necrotic/oncotic cell death. Proteomics analyses revealed that cytoprotection is associated with the increased synthesis of a select number of proteins, including retinol binding protein (RBP), actin, and glucose-regulated protein 78 (Grp78). We subsequently confirmed that all-trans-retinoic acid (ATRA) replicates DDM-PGE2-mediated cytoprotection against ROS-mediated cell death in LLC-PK1 and HK2 cells. ATRA also protected Fischer rats against p-aminophenol-induced kidney necrosis, as determined by a marked reduction in urinary enzyme leakage. In an ischemia reperfusion injury (IRI) mouse model, a single dose of ATRA (1mg/kg, i.p.) given 3-6 h prior to IRI also provided cytoprotection, with improved renal function, including reductions in BUN, serum Cr, reduced tubular necrosis scores, decreases in neutrophil infiltration and in renal proinflammatory cytokine TNF-α mRNA abundance (all at 24 h after reperfusion). Furthermore, at this therapeutic dose, ATRA induces renal Nrf2-responsive antioxidant HO-1 and NQO1 genes, as well as renal retinoic acid nuclear receptors RARα, RARβ2, RARγ2, and retinoid X receptors RXRα. Importantly, the transient induction of HO-1, mimicking renal preconditioning, is a key effector of ATRA-mediated renoprotection. In summary ATRA may provide an effective therapeutic strategy, offering protection against ROS induced renal injury caused by either ROS generating chemicals, or by those pathological conditions where ROS contribute to the disease process. (ES006694, ES016578).

PS 1275 TEMPOL AND TEMPOL-H PROTECT CARDIOMYOCYTES FROM NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI)-INDUCED MITOCHONDRIAL TOXICITY.

P. Nguyen[1], Y. Liu[1], J. B. Mitchell[2] and M. C. Poirier[1]. *[1]Carcinogen-DNA Interactions Section, LCBG, National Cancer Institute, National Institutes of Health, Bethesda, MD and [2]Tumor Biology Section, RBB, National Cancer Institute, National Institutes of Health, Bethesda, MD.*

NRTIs are essential components of the successful antiretroviral combination therapies used for treatment of HIV-1. However, during long-term therapy NRTIs damage heart mitochondria, thus limiting clinical use of these drugs. Consequently, reducing NRTI-induced mitochondrial toxicity may benefit HIV-1-infected patients. In these studies we have used H9c2 rat cardiomyocyte cultures to examine mitochondrial toxicity induced during long-term NRTI exposure. To evaluate intervention we have used the stable free radical Tempol and its metabolite Tempol-H. These compounds are cyclic nitroxides with antioxidant properties. H9c2 cells were cultured for 4 passages in the presence of 150 μM Zidovudine (AZT) and 150 μM Didanosine (ddI), and cytotoxicity was measured by Incucyte in the presence and absence of 100 μM Tempol and 100 μM of its metabolite Tempol-H. Maximal oxidative capacity was evaluated by Seahorse XF24, which measures oxygen consumption rate (OCR). At 48 hr of growth, for cells exposed for 4 passages, the cell survival for cells treated either with AZT/ddI or AZT/ddI plus Tempol or Tempol-H was 50% of that in the unexposed controls; addition of Tempol or Tempol–H did not restore cell survival. At passage 4 the maximal uncoupled OCR of cells exposed to AZT/ddI was about 50% of the unexposed controls, and incubation with Tempol or Tempol-H restored the maximal uncoupled OCR capacity to the level of the unexposed controls. The extent of restoration was statistically significant ($p<0.05$). Currently, we are evaluating underlying mechanisms related to the effect of Tempol and Tempol-H as mitochondrial protective agents.

PS 1276 NOVEL FUNCTIONS OF ALDH7A1 AGAINST HYPEROSMOTIC STRESS.

C. Brocker[1], M. Cantore[2], P. Failli[2] and V. Vasiliou[1]. *[1]Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO and [2]Department of Pharmacology, University of Florence, Florence, Italy.*

Mammalian aldehyde dehydrogenase 7A1 (ALDH7A1) is homologous to plant ALDH7B1 which protects against various forms of stress such as salinity and dehydration. In humans, deleterious mutations in ALDH7A1 are responsible for pyridoxine-dependent and folinic acid-responsive seizures. In previous studies, we have shown that ALDH7A1 protects against hyperosmotic stress by generating the osmolyte betaine from betaine aldehyde. In this study, we show that ALDH7A1 also protects cells from hyperosmotic stress by metabolizing reactive aldehydes and/or scavenging superoxide radicals. Human ALDH7A1 expression in Chinese hamster ovary (CHO) cells attenuated osmotic stress-induced cell death caused sodium chloride treatment. Moreover, MTT and SRB assays revealed that protein expression significantly protected cells from treatment with oxidants and lipid peroxide-derived reactive aldehydes, including hydrogen peroxide (H2O2), hexanal and 4-hydroxy-2-nonenal (4-HNE). SOD-inhibitable superoxide production was significantly reduced in ALDH7A1 expressing cells treated with sodium chloride. Furthermore, superoxide anion detection via dihydroethidium (DHE) staining also indicated decreased production in overexpressing cell lines. TBARS assays revealed that the decrease in superoxide production was accompanied by a decrease in lipid peroxidation. Hyperosmotic stress-induced caspase 3 activation was also significantly decreased in ALDH7A1 expressing cells indicating expression attenuates apoptosis. In conclusion, ALDH7A1 is a novel ALDH that protects against both hyperosmotic and oxidative stress by reducing superoxide production and removing toxic lipid peroxidation-derived aldehydes.

PS 1277 PROTECTIVE ROLE OF ATP BINDING CASSETTE TRANSPORTER ABCB6 IN ARSENIC TOXICITY.

H. Chavan, M. Oruganti and P. Krishnamurthy. *Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Arsenic is an inorganic carcinogen and a major environmental health concern. Arsenic damages biological systems through multiple mechanisms including the generation of reactive oxygen species. The ATP binding cassette transporter ABCB6 is expressed at high levels in arsenic resistant cells and ABCB6 is also demonstrated to protect cells against oxidative stress. In the present study we evaluated the significance of ABCB6 expression to arsenic toxicity in vivo in mice and in vitro in cell culture. Arsenite induces ABCB6 expression in a dose dependent manner both in mice fed sodium arsenite in drinking water and in vitro in cells exposed to sodium arsenite. However, arsenite induced ABCB6 expression is not mediated by the redox sensitive transcription factor Nrf2. We also demonstrate that knockdown of ABCB6 expression using siRNA sensitizes K562 and Hep3B cells to arsenite-induced toxicity. In contrast, stable over-expression of ABCB6 conferred a strong survival advantage and demonstrated reduced arsenite induced oxidative stress. Collectively these results, obtained by both loss of function and gain of function analysis, suggest that ABCB6 expression in response to sodium arsenite might be an endogenous protective mechanism activated to protect cells against arsenite induced oxidative stress.

PS 1278 ARSENIC ACTIVATES THE NRF2 ANTIOXIDANT RESPONSE PATHWAY *VIA* AUTOPHAGY.

A. Lau and D. D. Zhang. *Pharmacology and Toxicology, University of Arizona, College of Pharmacy, Tucson, AZ.*

The Nrf2 signaling pathway is an endogenous defense mechanism that protects against environmental insults, such as arsenic, by controlling the expression of many cellular protective proteins. Nrf2 is regulated by Keap1-Cul3-E3 ubiquitin ligase mediated ubiquitination and degradation. Upon induction of the Nrf2 pathway, the activity of the E3 ubiquitin ligase is compromised through the modification of cysteine residues in Keap1, resulting in the stabilization and activation of Nrf2. Previous research has demonstrated that arsenic activates the Nrf2 pathway in a cysteine-independent manner; however, this novel mechanism has yet to be elucidated. Recently, we identified a non-canonical cysteine-independent mechanism of Nrf2 activation, where p62, a substrate protein that has been shown to play a role in autophagy, sequesters Keap1 into aggregates. This hinders the activity of the Keap1-Cul3-E3 ubiquitin ligase and allows Nrf2 to escape degradation and trans-activate downstream genes. Our preliminary evidence suggests that arsenic induces autophagy causing the upregulation of p62 and the subsequent aggregation of Keap1 to activate the Nrf2 pathway. Immunoblot analyses using antibodies specific for p62 and LC3, a protein marker for autophagy, showed that HEK293 cells treated for 24 hours with 150nM, 250nM, 500nM, 1μM or 2μM of arsenic increased p62 protein levels and induced cleavage of LC3 in a dose-dependent manner. Additionally, live cell imaging of an immortalized baby mouse kidney cell line stably expressing EGFP-LC3 demonstrated that arsenic induced the formation of autophagosomes. Next, to confirm that arsenic induces autophagy, electron micrographs were taken of HEK293 cells treated with low doses of arsenic for 24 hours, which showed the formation of double-membrane autophagosomes in the cytoplasm. Collectively, these findings demonstrate that arsenic induces autophagy and offers a novel mechanism by which arsenic activates the Nrf2 pathway.

PS 1279 HISTONE DEACETYLASE 6 ASSOCIATES WITH THE POLYSOME DURING ARSENITE-INDUCED STRESS.

K. Kappeler, J. Zhang and Q. Chen. *University of Arizona, Tucson, AZ.*

Histone deacetylases 6 (HDAC6) is a cytoplasmic enzyme that has been shown to regulate cell migration, degradation of misfolded proteins, cell adhesion and immune responses. Reported targets of HDAC6 deacetylation include alpha-tubulin,

cortactin and Hsp90. HDAC6 interacts with Ras-GTPase-activating protein SH3 domain-binding protein 1 (G3BP) under stress conditions, facilitating protein locomotion and stress granule formation. To address whether HDAC6 mediates cellular response to Arsenite stress, we used HaCat keratinocytes for treatment with Sodium Arsenite. Sodium Arsenite induced a morphological change characteristic of cellular condensing. Immunocytochemistry experiments revealed HDAC6 relocalized to the perinuclear space during Sodium Arsenite-induced stress within 30 minutes. There was no difference in the overall level of HDAC6 protein with various doses of Sodium Arsenite by Western blot analyses. Perinuclear localization of HDAC6 correlates with a dose dependent increase of association with polysomes. These results suggest HDAC6 interacts with ribosomal proteins. Several ribosomal proteins were examined by immunocytochemistry to determine co-localization with HDAC6. We found that Sodium Arsenite caused HDAC6 to co-localize with L36a ribosomal proteins. Immunoprecipitation experiments indicate physical interaction of HDAC6 with ribosomal protein L36a. This study demonstrated that HDAC6 is recruited to the polysomes during stress and interacts specifically with ribosomal protein L36a. This finding may provide a better understanding of ribosomal regulation during stress through post-translation modifications of specific ribosomal subunits.

PS 1280 POST-TRANSLATIONAL MODIFICATION AND REGULATION OF GLUTAMATE CYSTEINE LIGASE BY THE α, β-UNSATURATED ALDEHYDES ACROLEIN AND 4-HYDROXYNONENAL.

D. S. Backos[1], K. S. Fritz[1], J. R. Roede[2], D. R. Petersen[1] and C. C. Franklin[1]. *[1]Department of Pharmaceutical Sciences, University of Colorado Denver, Denver, CO and [2]Department of Medicine, Pulmonary Division, Emory University, Atlanta, GA.*

Acrolein and 4-hydroxynonenal (4-HNE) are cytotoxic α,β-unsaturated aldehydes produced via lipid peroxidation. These reactive aldehydes can alter protein function by direct covalent modification of nucleophilic amino acid residues (Cys, His, Lys, (Arg)). Mammalian cells possess a number of antioxidant defense mechanisms to counteract the deleterious effects of oxidative stress and reactive aldehydes. The glutathione (GSH) antioxidant defense system plays a critical role in maintaining cellular redox homeostasis and detoxification of reactive aldehydes via GSH conjugation. The first and rate-limiting step in GSH biosynthesis is catalyzed by glutamate cysteine ligase (GCL), a heterodimeric holoenzyme composed of a catalytic (GCLC) and modulatory (GCLM) subunit. In this study, purified recombinant proteins were utilized to demonstrate that GCLC and GCLM are targets for post-translational modification by acrolein and 4-HNE. While both aldehydes inhibited GCL holoenzyme formation and activity, they differentially affected monomeric GCLC activity. 4-HNE activated GCLC, while acrolein had a biphasic effect on GCLC enzymatic activity. Residue masking studies indicated that Cys and Lys residues were the primary targets of adduction on both GCL subunits. Furthermore, mass spectrometry analysis identified several residues that were differentially modified by acrolein and 4-HNE, and may be functionally relevant based on in silico molecular modeling. In aggregate, these findings demonstrate that acrolein and 4-HNE can alter GCLC activity and GCL holoenzyme formation and activity via direct post-translational modification of the GCL subunits in vitro. Within a cellular context, this novel post-translational regulation of GCL activity could significantly affect cellular redox homeostasis and GSH-dependent detoxification during periods of oxidative stress.

PS 1281 IDENTIFICATION OF HYDROIMIDAZOLONE AND ARGPYRIMIDINE ADDUCTS AS GLYCOOXIDATIVE BIOMARKERS IN TYPE 2 DIABETIC SUBJECTS.

O. Kinsky[1], M. J. Kimzey[1], H. N. Yassine[2], C. S. Stump[2], G. Tsaiprailis[1], T. J. Monks[1] and S. S. Lau[1]. *[1]Southwest Environmental Health Sciences Center, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ and [2]College of Medicine, University of Arizona, Tucson, AZ.*

Oxidative stress and advanced glycation (glyco-oxidation) produce reactive dicarbonyls such as methylglyoxal (MG), 3-deoxyglucosone and glucosone. Dicarbonyls primarily react with arginine residues, resulting in irreversibly modified proteins as a consequence of the net loss of positive charge via hydroimidazolone and, to a lesser extent, argpyrimidine ring formation. These dicarbonyl adducts on human serum albumin (HSA) may be useful as candidate biomarkers for oxidative stress-related diseases. We employed multiple-reaction monitoring (MRM) on 12 synthetic HSA peptides, with three dicarbonyl modifications each, to generate a transition list to quantitate relative modification at each site. In addition to MRM, we determined whether MG-modified HSA was detectable in the urine of type II diabetes patients (T2D) with macroalbuminuria. Digests of the dicarbonyl modified HSA revealed R410 as the dominant hotspot in vitro for MG, 3-deoxyglucosone, and glucosone. In addition to R410, both R257 and R186 were found to be hotspots as well, with R186 being the least reactive of the three sites. Western blot analyses using anti-MG antibody on urine from T2D patients with macroalbuminuria indicated detectable levels of MG-related modifications on several proteins, primarily albumin. From the MRM data, we have determined that quantitation of glyco-oxidation from reactive dicarbonyls is suitable using tandem LC-MS/MS analysis. In summary, using two complementary approaches we have identified MG-modified HSA by MRM in plasma and western blot analysis in urine from T2D patients with macroalbuminuria. MG-protein modifications of plasma and/or urinary proteins may serve as markers of diabetic complications. (P30ES006694, R24DK083948, T32 ES016652, ABRC 10-100).

PS 1282 IDENTIFICATION AND CHARACTERIZATION OF 4-HNE MODIFIED LIVER FATTY ACID BINDING PROTEIN IN A RODENT MODEL OF EARLY ALCOHOLIC LIVER DISEASE.

R. L. Smathers, K. S. Fritz, J. J. Galligan, C. T. Shearn and D. R. Petersen. *Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Chronic ethanol consumption is a prominent cause of liver disease and is responsible for significant morbidity and mortality throughout the Western world. Among the predictable and prominent histologic abnormalities resulting from ethanol ingestion is hepatosteatosis (fatty liver). The mechanisms behind the observed fluctuations in lipid homeostasis include, but are not limited to, increased hepatocellular synthesis and decreased β-oxidation of fatty acids, as well as impairment of cellular trafficking and secretion of hepatic lipids. Using a proteome-wide scan of cellular extracts prepared from livers of mice ingesting alcohol for 6 weeks, we identified liver fatty acid binding protein (L-FABP) as a target for modification by the aldehydic product of lipid peroxidation, 4-hydroxynonenal (4-HNE). Utilizing LC-MS/MS and MALDI-TOF/TOF mass spectrometry, residues susceptible to modification by 4-HNE were identified at H43, C69, K31 and K57 from purified recombinant mouse L-FABP (rL-FABP). Saturation isothermic analysis of 4-HNE-treated rL-FABP revealed significant reductions in maximal binding and markedly altered high- and low-affinity dissociation constants for the fluorescent probe, 1-anilinonaphthalene-8-sulfonic acid (ANS). Stability of adducted protein was also analyzed via thermal denaturation, demonstrating the detrimental effects of 4-HNE modification to L-FABP. Significant changes in the compartmental expression of L-FABP, expression and activity of peroxisome proliferator activated receptor-alpha (PPAR-α), and ubiquitinated proteins were observed in ethanol-treated mice. Immunohistochemical evaluation of liver sections also revealed considerable changes in lobular expression and localization of L-FABP. Collectively, these data demonstrate in vivo and in vitro modification of L-FABP by 4-HNE; suggesting L-FABP as a potential target for study of the initiation and progression of alcoholic liver disease. (R37 NIH/AA009300; NIH/NIAAA 5F31 AA18898-02)

PS 1283 EXTENSIVE OXIDATIVE PROTEIN MODIFICATIONS OBSERVED IN HUMAN PLASMA AND CANDIDATE BIOMARKERS OF SYSTEMIC CHRONIC INFLAMMATORY AND OXIDATIVE STRESS.

X. Zhang, M. A. Gritsenko, B. Webb-Robertson, K. M. Waters, D. J. Bigelow, W. Qian, J. G. Pounds and J. M. Jacobs. *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA.*

Smoking and obesity are two of the most important, yet preventable risk factors for human morbidity and mortality. Chronic inflammation and oxidative stress appears to be the unifying mechanism underlying the interaction of these risk factors with the genome, resulting in a variety of chronic human diseases. We are identifying potential protein biomarkers of oxidative stress in human populations that accurately and quantitatively reflect an individual's exposure/response to environmental stressors. A subset of plasma samples were analyzed from a cohort of 500 exposed to main-stream tobacco smoke or never-smoked with BMI above 35 or below 25. The four representative pooled groups included high BMI smoker and non-smokers, and low BMI smoker and non-smokers. Individual human plasma in each group was pooled, depleted, digested with trypsin, with the resulting peptides fractionated using SCX chromatography and subsequently analyzed via an LTQ-Orbitrap mass spectrometer for a total of 100 LC-MS/MS analyses. Data were searched using X!Tandem against the human International Protein Index database resulting in 9908 confident peptide identifications corresponding to 1306 proteins. Searches for 5 different oxidative modifications on tyrosine residues and 2 on cysteine residues resulted in >1500 unique modified peptides. Extensive oxidation of plasma proteins was observed, some with multiple modifications. Dopa-quinone and cysteine sulfonic modifications of AT and alpha-2M were of interest because oxidative modification of these proteins is known to contribute to disease risk and development. The differential abundance of modified peptides from several pro-

teins (e.g. C3, 4, and 5, APOB, VDBP, alpha-1 ACT, and ITIH1) were highly effective in classifying plasma samples to phenotype. These proteins should provide insights into systemic chronic inflammation, serve as biomarkers, or new drug targets for intervention. Supported by NIEHS U54016105

PS 1284 EXTENSIVE OXIDATIVE PROTEIN MODIFICATIONS OBSERVED IN HUMAN PLASMA AND CANDIDATE BIOMARKERS OF SYSTEMIC CHRONIC INFLAMMATORY AND OXIDATIVE STRESS.

B. Webb-Robertson, X. Zhang, K. M. Waters, W. Qian, J. M. Jacobs, D. J. Bigelow, R. Corley and J. G. Pounds. *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA.*

Smoking and obesity are two of the most important, yet preventable risk factors for human morbidity and mortality. Chronic inflammation and oxidative stress appears to be the unifying mechanism underlying the interaction of these risk factors with the genome, resulting in a variety of chronic human diseases. We are identifying potential protein biomarkers of oxidative stress in human populations that accurately and quantitatively reflect an individual's exposure/response to environmental stressors. A subset of plasma samples were analyzed from a cohort of 500 exposed to main-stream tobacco smoke or never-smoked with BMI above 35 or below 25. Eight plasma samples per group representing high BMI smoker and non-smokers, and low BMI smoker and non-smokers were Individual human plasma were depleted, digested with trypsin and analyzed via an LTQ-Orbitrap mass spectrometer in triplicate. Peptides were identified by accurate mass and time (AMT) tags. 4616 peptides passed quality control and were analyzied by ANOVA and G-test for statistical significance to experimental groups. 133 proteins are significant for either smoking status or BMI. 94 of these proteins had at least one oxidatively modified proteins. Statistical and graphical analysis revealed that the abundance of unmodified peptides are typically similar across the four groups while the abundance of modified peptides is group specific. This trend is particularly noted for the proteins Complement factor D, Plasminogen-like B2, and Fibrinogen. The differential abundance of modified 0and unmodified peptides/proteins were highly effective in classifying plasma samples to phenotype. These proteins should provide insights into systemic chronic inflammation, serve as biomarkers, or new drug targets for intervention. Supported by NIEHS U54016105

PS 1285 DEVELOPMENT OF MALDI-TOF METHODOLOGY FOR SELECTIVE MS-ANALYSIS AND IMAGING OF CARDIOLIPINS IN LIPID EXTRACTS AND TISSUES.

L. J. Sparvero[1, 3], A. Amoscato[1, 3], B. R. Pitt[1, 3], H. Bayir[2, 3] and V. E. Kagan[1, 3]. *[1]Center for Free Radical and Antioxidant Health, Environmental and Occupational Health, Pittsburgh, PA, [2]Critical Care Medicine, Safar Center for Resuscitation Research, Pittsburgh, PA and [3]University of Pittsburgh, Pittsburgh, PA.*

Cardiolipin (CL) is an anionic phospholipid that plays a key role in mitochondrial electron transport but is also highly susceptible to oxidative stress. The molecular diversity of endogenous cardiolipins in some tissues and the low abundance of its individual species, particularly oxidized derivatives, makes in situ analysis and imaging by MALDI-Mass Spectrometry (MALDI-MS) challenging. The goal of this study was to develop a method to selectively analyze CL using existing CL probes in a novel way, to decrease CL mass spectral complexity. Nonyl Acridine Orange (NAO) is a commonly used fluorescent stain for cardiolipin. We have used NAO as a novel MALDI matrix, and have found that it allowed detection of anionic phospholipids, including cardiolipin in the negative ion mode. With careful laser tuning, NAO induced prompt decay of cardiolipin selectively - but not other phospholipids - at very high efficiencies. From these selective prompt decay products, much information about the structure of cardiolipin can be determined. Pseudo-MS^3 is possible on a MALDI-TOF instrument by doing MS/MS on these products of cardiolipin. We have also extended these findings to include oxidized species of CL's and have determined that NAO also induced prompt decay of oxidized CL. Prompt fragments are seldom seen with dihydroxy-benzoic acid (DHB) which has been employed as a standard matrix for lipid analysis in the negative ion mode. Our results indicate that NAO can be utilized as a discerning matrix for the assessment of CL in lipid extracts from cells and the distribution of CL - particularly in tissues with a high diversity of CL molecular species and their oxidation products - by taking full advantage of the unique ability to induce prompt decay of this phospholipid with NAO.
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PS 1286 PROTEOMIC IDENTIFICATION OF NITRATED PROTEINS IN THE SPLEEN OF ANILINE EXPOSED RATS.

X. Fan[1], J. Wang[1], K. Soman[2], G. Ansari[1, 2] and M. Khan[1]. *[1]Pathology, University of Texas Medical Branch, Galveston, TX and [2]BMB, University of Texas Medical Branch, Galveston, TX.*

Aniline exposure is associated with toxicity to the spleen. However, mechanisms by which aniline elicits splenotoxic response are not well understood. Earlier we have shown that aniline exposure leads to increased nitration of proteins in the spleen. However, nitrated proteins remain to be identified and characterized. Identification of nitrated proteins is important because nitration of proteins could result in their inactivation, increased degradation and altered tyrosine phosphorylation, and thus, could contribute to splenic toxicity. Therefore, the current study, using proteomic approaches (2D Western blot, MALDI TOF/TOF MS/MS and Progenesis SameSpots, etc.), was focused on identifying and characterizing nitrated proteins in the spleen of aniline exposed rats (0.5 mmol/kg/day for 30 days via drinking water). Aniline exposure led to increased tyrosine nitration of proteins, as determined by 2D Western blotting with specific anti-3-nitrotyrosine antibody, compared to the controls. The nitrated proteins were found in the molecular weight range from 27.7 to 123.6 kDa. A total of 37 nitrated proteins were identified in the protein extracts of aniline-treated and control spleens. Among them, 25 were found only in aniline-treated rats, 11 were present in both aniline-treated and control rats, while one nitrated protein was found in controls only. The nitrated proteins identified mainly represent skeleton proteins, chaperones, ferric iron transporter, enzymes, nucleic acids binding protein, and signaling and protein synthesis pathways. The increased nitration of proteins not only supports our previous findings, but more importantly, provides a global map to further investigate alterations in their structural and functional properties, and thus, a better understanding of the role of the protein nitration in splenic toxicity. Supported by NIH ES006476.

PS 1287 CIRCULATING LEVELS OF NITRATED PROTEINS ARE SUPPRESSED BY CIGARETTE SMOKING.

R. Zangar[1], H. Jin[1], B. Webb-Robertson[1], D. Bigelow[1], M. Scholand[2], J. Hoidal[2] and J. Pounds[1]. *[1]Pacific Northwest National Laboratory, Richland, WA and [2]Health Sciences Center, University of Utah, Salt Lake City, UT.*

Nitric oxide (NO) is a physiological regulator of endothelial function and hemodynamics. Oxidized products of NO can form nitrotyrosine in proteins, a general marker of nitrative stress. Cigarette smoking decreases exhaled NO, and the underlying mechanism may be important in cardiovascular toxicity. Even so, it is unclear if this decrease in exhaled NO is due to lower NO production or increased NO degradation to nitrating species. Since these two processes should have opposing effects on nitrotyrosine levels, our goal was to examine nitration levels in circulating proteins in order to gain insight into which of these processes predominates in smokers. A custom antibody microarray platform was developed to analyze the levels of 3-nitrotyrosine modifications on 24 proteins in plasma. Plasma samples from 458 individuals including non-smokers, former smokers, and active smokers with or without chronic obstructive pulmonary disease (COPD) were analyzed. Levels of nitrated plasma proteins were consistently lower in current and former smokers than in plasma from non-smokers; protein nitrotyrosine levels in individuals at risk for environmental cigarette smoke exposure was intermediate between smokers and non-smokers. These results are consistent with evidence that soluble cigarette-smoke components suppress endothelial NO synthase activity, leading to endothelial dysfunction and cardiovascular disease. In contrast, we observe an increase in nitrotyrosine levels in individuals with COPD that likely reflects an increase in inflammation. This study provides the first evidence that smoking has irreversible effects on endothelial production of nitric oxide in humans, and provides insight into how smoking could induce endothelial dysfunction and a long-term increase in the risk of cardiovascular disease. Supported by NIEHS Exposure Biology Program, U54 ES106015.

PS 1288 URINARY 8-OXO-DEOXYGUANOSINE IN SMOKERS: ASSOCIATIONS WITH RACE, SEX, AND MENTHOL SMOKING.

M. Misra and D. Ergle. *Lorillard Tobacco Company, Greensboro, NC.*

Several recent studies have found no significant differences in either biomarkers of exposure or biomarkers of potential harm in the blood and urine of menthol smokers compared to non-menthol smokers. Differential uptake or metabolism of cigarette smoke constituents can induce oxidative stress and lead to the oxidative modification of the guanine base of DNA. This modification results in the formation of

8-oxo-deoxyguanosine (8-oxo-dG), a hallmark biomarker for oxidative stress in biological systems. We conducted the current study to determine if levels of 8-oxo-dG in the urine of smokers were differentiated by race, sex, or the smoking of menthol cigarettes. Our study of 110 heavy smokers included 56 non-menthol smokers and 54 menthol smokers whose usual brands had similar yields (~ 10 mg "tar" machine-measured under the ISO protocol), comprised 63 female & 47 male subjects, and 28 black & 82 white participants. Individuals were allowed to smoke their usual brand ad libitum and averaged 31 cigarettes per day. There was no statistically significant difference in 8-oxo-dG levels between smokers of menthol (median, 96.1 (25th, 75.8; 75th, 141.2) pg/mg creatinine) and smokers of non-menthol cigarettes (105.1 (73.0, 155.2) pg/mg creatinine, $p = 0.25$). There was also no difference between menthol and non-menthol smokers when further segregated by race or by sex. Levels of urinary 8-oxo-dG were 14% higher among female smokers (105.6 (79.8, 165.7) pg/mg creatinine) compared to male smokers (92.8 (63.4, 142.6) pg/mg creatinine, $p < 0.05$), and 22% higher among white smokers (105.6 (81.2, 165.7) pg/mg creatinine) compared to black smokers (86.5 (63.4, 113.6) pg/mg creatinine, $p < 0.05$). Correlations between urinary biomarkers of exposure and levels of 8-oxo-dG demonstrated a modest association with NNAL ($r = 0.55$), trans-3'-hydroxycotinine ($r = 0.56$), and cotinine ($r = 0.51$). There was no apparent correlation with urinary nicotine ($r = 0.35$) or blood COHb ($r = 0.15$). This study findings indicate that urinary 8-oxo-dG levels were not differentiated by the smoking of menthol cigarettes.

PS 1289 IMBALANCED EPITHELIAL ANTI-VIRAL RESPONSES INDUCED BY CIGARETTE SMOKE (CS).

S. Tao and Y. Chen. *Pharmacology and Toxicology, School of Pharmacy, Tucson, AZ.*

Historically, bacteria have been considered to be the main infectious cause of the exacerbation of cigarette smoke (CS) related diseases such as chronic obstructive pulmonary disease (COPD). However, the wide use of sensitive detection method (e.g. PCR) has demonstrated that viruses are major contributors in almost 40% of outpatient COPD exacerbations and an even higher percentage of patients with severe exacerbations. Human Rhinovirus (RV) has been found to be a major viral pathogen to induce COPD exacerbations. But the underlying mechanism is not clear. Since airway epithelium is the primary encounter of CS exposure and also the main route of RV infection, we used human airway epithelial cell culture (both cell line and primary cell) as an in vitro model to examine the interaction between CS exposure and RV infection. Interestingly, pre-exposure to CS synergistically increased RV-induced pro-inflammatory cytokine production, while severely blocked the Interferon (IFN) dependent anti-viral defense. The latter effect led to a significant increase of RV production, which could further increase the inflammation and exacerbate the symptom of the existing disease as what would happen in the airways of COPD exacerbation.

PS 1290 NITRITE AND METHEMOGLOBIN FORMATION UPON NO2 EXPOSURE MAY TRIGGER OXIDANT STRESS AND CARDIOVASCULAR DYSFUNCTION. POSSIBLE MECHANISM FOR TRAFFIC-INDUCED ADVERSE EFFECTS.

C. Monteil[1], C. Crochemore[1], V. Keravec[2], D. Preterre[2] and J. Morin[1]. [1]*U644, INSERM, ROUEN, France and* [2]*CERTAM, CERTAM, Saint Eienne du Rouvray, France.* Sponsor: R. Forster.

Epidemiological study clearly associate atmospheric pollution and cardiorespiratory diseases. Diesel engine emissions are clearly designated as an important trigger. Beside particulate matter, NO2 seems to to be a major candidate for inducing tissular oxidant damage. The use of oxidation catalyst as a diesel engine emission after-treatment strategy proved to induce the highest oxidative damage on both in vitro and in vivo systems during direct aerosol exposure. The observed oxidative damage downstream oxidation catalyst closely correlates the NO2 concentrations but failed to correlate particulate matter concentrations. We used CPH as a spin probe to interact with potential ROS present in combustion emitted aerosols, CP* was then assayed by electron spin resonance ESR. The occurrence of CP* closely correlates aerosol NO2 concentrations but failed to correlate particulate matter. We demonstrate the occurrence of CP* formation during the exposure of cell culture medium for 60 min to a continuous flow of 5ppm NO2 which was inhibited by the presence of anti-oxidant agents like GSH, ascorbate, MEG or sodium dithionite. We also demonstrate the formation of nitrite and nitrate in the culture media exposed to 5ppm NO2. Nitrite is known to react with hemoglobin to form methemoglobin we thus exposed human and rat blood for 1 and 3 hours to a continuous flow of NO2 and then assayed for methemoglobin formation. A concentration and time dependent formation of methemoglobin was clearly evidenced from 3ppm NO2 in the atmosphere. We suggest that beside the GSH depletion and oxidative stress known to occur in lung tissue and BAL, gaseous NO2 when interacting with blood in the alveolar area may produce nitrite and methemoglobin. Both nitrite and methemoglobin formed upon NO2 exposure may trigger cardiovascular dysfunction and contribute to the mechanisms of traffic induced adverse effects.

PS 1291 PARTICULATE MATTER-INDUCED LIPID PROFILE ALTERATIONS IN HUMAN ENDOTHELIAL CELLS.

L. Yu[1] and A. Jeng[2]. [1]*Biomedical Sciences Program, Old Dominion University, Norfolk, VA and* [2]*Community and Environmental Health, Old Dominion University, Norfolk, VA.*

Epidemiological and animal data have shown that exposure to ambient particulate matter (PM) lead to enhanced atherosclerosis. PM can induce lipid peroxidation and form bioactive lipids, which may be involved with the pathogenesis of atherosclerosis. However, data on assessment of oxidized lipid formation induced by PM are very limited due to low resolution of mass spectrometry methods. Taking advantage of available Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR-MS) with 12 Tesla at Old Dominion University, this study aims to assess lipid peroxidation induced by PM at 2.5 μm and how it alters the lipid profile and oxidizes lipids in human coronary artery endothelial cells. The endothelial cells were exposed to PM2.5 as a function of two variables: the dose of PM2.5 and the duration time of exposure. Then, lipid peroxidation in cells was assessed, and lipids were extracted for lipid species identification. After PM2.5 exposure, malondialdehyde levels increased and correlated with reactive oxygen species levels. The analysis of spectrum data from FT-ICR-MS showed changes of cellular lipid profile in the exposed groups as compared to a control. Van Krevelen diagrams revealed PM-exposed groups had cluster molecules, defined as lipids according to the compound classes. Kendrick mass defect analysis was used to differentiate the exact mass of the functional COO group; the homologous series falling on the same line suggested oxidation was occurring. In conclusion, PM2.5 caused an oxidation pathway to form oxidative lipids in cells. This study has advanced the analytical ability to identify lipids and develop novel data analysis approaches that are applicable in revealing oxidative lipid formation induced by PM2.5.

PS 1292 HYDROXYL RADICAL FORMATION BY REDOX CYCLING OF QUINONES IN DIESEL EXHAUST PARTICLES.

R. G. Udasin[1], K. C. Fussell[1], Y. Wang[2], V. Mishin[3], D. E. Heck[4], D. L. Laskin[1, 3] and J. D. Laskin[1, 2]. [1]*Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ,* [2]*Environmental & Occupational Medicine, UMDNJ-RW Johnson Med. Sch, Piscataway, NJ,* [3]*Pharmacology & Toxicology, Rutgers University, Piscataway, NJ and* [4]*Department of Environmental Health Science, New York Medical College, Valhalla, NY.*

Epidemiologic studies have demonstrated a strong association between air pollution and pulmonary morbidity and mortality. Of particular concern are fine particulate matter (PM). Diesel exhaust particles (DEP) are a significant source of fine PM in the atmosphere. Typically containing a carbon core that absorbs many organic compounds including polycyclic aromatic hydrocarbons, aldehydes and quinones, DEP are known to stimulate both macrophages and bronchial epithelial cells to generate reactive oxygen species (ROS). Quinones are of particular interest because of their ability to redox cycle and generate toxic hydroxyl radicals. In the present studies, redox cycling of three quinones in DEP [1,2-naphthoquinone (1,2-NQ), 1,4-naphthoquinone (1,4-NQ), and 9,10-phenanthrenequinone (PAQ)] was compared in enzyme assays using rat liver microsomes. Hydroxyl radicals were measured using terephthalate (TPT), a fluorescent hydroxyl radical trap. Quinone redox cycling was NADPH-dependent and generated both superoxide anion and hydrogen peroxide. Hydroxyl radicals were only formed in the presence of iron. PAQ was efficient in generating hydroxyl radicals (Km = 0.37 μM, Vmax = 1.6 nmol 2-OH TPT/min/mg microsomal protein), followed by 1,4-NQ (Km = 0.87 μM, Vmax = 2.4) and 1,2-NQ (Km = 2.7 μM, Vmax = 4.2). Hydroxyl radical formation was inhibited by diphenyleneiodonium, a flavoenzyme inhibitor, indicating that cytochrome P450 or cytochrome b5 reductase mediated redox cycling. Antioxidants such as DMSO, GSH, resveratrol and α-tocopherol blocked hydroxyl formation. These data indicate that DEP-associated quinones are effective redox cycling agents. Formation of cytotoxic ROS such as hydroxyl radicals have the potential to contribute to PM-induced lung injury. Support: AR055073, ES004738, CA093798, CA132624 and ES05022.

PS 1293 TRANSFORMING GROWTH FACTOR BETA MEDIATES OZONE-INDUCED AIRWAY WALL FIBROSIS.

R. Liu[1], A. Katre[1], C. Ballinger[1], H. Akhter[1], M. Fanucchi[1], D. Kim[2] and E. Postlethwait[1]. [1]*University of Alabama at Birmingham, Birmingham, AL and* [2]*Ewha Womans University, Seoul, Republic of Korea.*

Background: Ozone (O3), a commonly encountered environmental pollutant, has been shown to induce pulmonary fibrosis in different animal models; the underlying molecular mechanism, however, remains elusive. Methods: Six to eight week old male C57BL mice were exposed to a cyclic O3 exposure protocol consisting of 2 days of filtered air and 5 days of O3 exposure (0.5 ppm, 8 hrs/day) for 5 and 10 cycles with or without intra-peritoneal injection of IN-1233, an inhibitor of type 1 receptor of transforming growth factor beta (TGF-β), the most potent profibrogenic cytokine. Results: The results showed that O3 exposure for 5 or 10 cycles increased the TGF-β protein level in the bronchoalveolar lavage fluid (BALF), associated with an increase in the expression of plasminogen activator inhibitor 1 (PAI-1), a TGF-β responsive gene which plays a critical role in the development of fibrosis. Cyclic O3 exposure also increased the deposition of collagens and alpha smooth muscle actin (alpha-SMA) within airway walls. However, these fibrotic changes were not overt until after 10 cycles of exposure. We further showed that treatment of mice with IN-1233 suppressed O3-induced PAI-1 expression and fibrotic changes. Conclusion: Our data demonstrate for the first time that activation of the TGF-β signaling pathways mediates O3-induced lung fibrotic responses in vivo.

PS 1294 ANGIOTENSIN II PLAYS A CRITICAL ROLE IN DIABETIC LUNG FIBROSIS *VIA* ACTIVATION OF NADPH OXIDASE-MEDIATED NITROSATIVE DAMAGE.

L. Cai[1, 2], J. Yang[2, 1], Y. Tan[1] and L. Miao[2]. [1]*Pediatrics, University of Louisville, Louisville, KY and* [2]*Second Hospital of Jilin University, Changchun, China.*

Diabetic patients have a high risk of pulmonary disorders that are usually associated with restrictive impairment of lung function, suggesting a fibrotic process. Human pathological examination on the hung from diabetic patients supports the existence of lung fibrosis. However, it is remains unclear whether diabetes is able, and how to induce lung fibrosis. The present study was undertaken to define whether and how diabetes causes lung fibrosis. Lung samples from streptozotocin-induced type 1 diabetic mice, spontaneously developed type 1 diabetic OVE26 mice, and their age-matched controls, were investigated with histopathological and biochemical analysis. Signaling mechanisms were also explored with cultured normal human lung fibroblasts in vitro. In diabetes, histological examination with Sirius-red staining and H & E staining showed significant fibrosis, along with massive inflammatory cell infiltration. Lung fibrosis and inflammation were confirmed by real time PCR and Western blotting assays for the increased fibronectin, CTGF, PAI-1 and TNF-α mRNA and protein expressions. Diabetes also significantly increased NADPH oxidase (NOX) expression and protein nitration, along with up-regulation of angiotensin II (Ang II) and its receptor expression. In cell culture, exposure of lung fibroblasts to Ang II increased CTGF expression in a dose- and time-dependent manner, which could be abolished by inhibition of superoxide, NO, and peroxynitrite accumulation. Furthermore, chronic infusion of Ang II to normal mice at a subpressor dose induced diabetes-like lung fibrosis, and Ang II receptor AT1 blocker (losartan) abolished the lung fibrotic and inflammatory responses in diabetic mice. These results suggest that Ang II plays a critical role in diabetic lung fibrosis, which is caused by NOX activation-mediated nitrosative damage.

PS 1295 THE ROLE OF NITRIC OXIDE SYNTHASE IN PARAQUAT-INDUCED OXIDATIVE STRESS IN A SKELETAL MUSCLE CELL LINE.

P. Venkatakrishnan, S. Panda, L. Roman and B. S. Masters. *Biochemistry, University of Texas Health Science Center, San Antonio, TX.*

Nitric Oxide Synthase (NOS) is a heme-containing flavoenzyme that catalyzes the conversion of L-arginine to L-citrulline, and NO· is formed as the co-product. The function of NO· varies depending on the NOS isoform and the location at which it is being produced. NO· produced by nNOS facilitates neurosignaling, by eNOS, vasodilation, and iNOS, cytoprotection from foreign agents. nNOSμ, a splice variant form of nNOS containing an extra 34 amino acid insert between exon 16 and 17, was identified to be present in the skeletal muscle and heart. In skeletal muscle, nNOS is complexed with the Dystrophin-Glycoprotein Complex (DGC) where it is bound through α-syntrophin. Although the function of this NOS isoform in the skeletal muscle is poorly understood, nNOS membrane localization with DGC is critical for vasoconstriction and in case of Duchenne Muscular Dystrophy, nNOS is found to be displaced from the sarcolemma. In order to understand the role of nNOSμ in the regulation of antioxidants in skeletal muscle during aging, we examined the functional significance of nNOSμ in C2C12 myoblast cell line under conditions of oxidative stress using paraquat(PQ). C2C12 cells were subjected to serum starvation and thus allowed to differentiate to form myotubes. Myotubes were pretreated with *L*-NAME(3 mM), an nNOS inhibitor, for 45 mins and later with PQ(100 μM) for various time points (2, 4, 8, 12, 24 and 48, 72 hrs) with appropriate controls. Morphological changes of myotubes clearly indicate that *L*-NAME has a protective effect on PQ-induced damage at intermediate time points. Cell lysates were collected at these time points for Western Blotting to probe for β-dystroglycan, nNOSα (also known as nNOS), nNOSμ and oxidative stress markers such as peroxyredoxin 6. In addition, the similarity and differences in the enzymatic activity in terms of electron transfer efficiency between nNOSα and nNOSμ were studied.

PS 1296 GENERATION OF ACETYL RADICAL AND SINGLET OXYGEN BY REACTIVE ALPHA-OXOALDEHYDES LINKED TO AGING AND DIABETES.

J. Massari[1] and E. J. Bechara[1, 2]. [1]*Ciências Exatas e da Terra, Universidade Federal de São Paulo, Diadema, São Paulo, Brazil and* [2]*Biochemistry, São Paulo University, São Paulo, São Paulo, Brazil.* Sponsor: S. Berlanga Barros.

Diacetyl (DA) - a food contaminant, methylglyoxal (MG) - an endogenous toxin in diabetes, and glyoxal (GO) – a metabolite implicated in ageing, are increasingly more reactive than monocarbonyls regarding nucleophilic additions to proteins and DNA. We have recently reported that DA e MG undergo attack by peroxynitrite, a strong biological oxidant and nucleophile, in phosphate buffer pH 7, followed by oxidation by oxygen. The reaction putatively involves the intermediacy of a nitrosoperoxo adduct, followed by homolysis to an oxyl radical, β-scission to acetyl radical, and ultimately formation of acetate (from DA) or acetate plus formate (from MG. These reactions could be coupled to radical acetylation of added amino acids and nucleobases, leading us to hypothesize that they may be involved in post-translational protein modifications and mutagenesis. We now show that at identical experimental conditions GO reacts with peroxynitrite to yield mainly formate at rates at least one order of magnitude higher than MG. The novelty here, predicted by assuming subsequent formation of a formyl radical from GO, dioxygen insertion, and Russell mechanism reaction, is the detection of the singlet molecular oxygen monomol light emission at 1270 nm during the reaction. Accordingly, the infra-red emission area increases upon raising the concentration of peroxynitrite, is suppressed upon addition of singlet quenchers (azide, His), is enhanced in deuterated buffer, and is not observed with DA, a source of acetyl, but not formyl radical. Chemical trapping of singlet oxygen using anthracene-9,10-divinylsulfonate was also performed. Our studies may add insights to detail the molecular events underlying nitrosative, oxidative and carbonyl unbalance in some disorders. Support: FAPESP, CNPq, INCT Redoxoma.

PS 1297 CARBONYLATION AND INACTIVATION OF MITOCHONDRIAL COMPLEX I DURING KAINATE-INDUCED EPILEPTOGENESIS.

K. Ryan and M. Patel. *Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO.*

Nearly 1% of the population suffers from epilepsy. Acquired epilepsies such as temporal lobe epilepsy (TLE) resulting from trauma, chemical exposure or infections comprise a majority of epilepsy cases. Like many progressive disorders, mitochondrial dysfunction and oxidative stress are linked to the development of TLE. A single injection of kainate, an excitotoxic agent, results in acute excitotoxicity and chronic development of spontaneous seizures via a process termed epileptogenesis. We have previously shown increased mitochondrial-specific reactive oxygen species (ROS) production, mitochondrial DNA damage and decreases in mitochondrial redox status in the rat hippocampus in the kainate model of epileptogenesis (Jarrett S et al 2008; Liang L et al 2006). We hypothesize that Complex I (CI) dysfunction may be the primary cause of mitochondrial inhibition and contribute to seizures: increasing evidence suggests that ROS-induced protein post-translational modifications are a contributing factor. The goal of this study was to determine if the mechanism of CI dysfunction is related to oxidative modification of the 75kDa subunit (Ndusf1) within the complex. Rats were injected with kainate or vehicle and monitored by video and EEG for seizure activity for 6 weeks. Mitochondrial CI activity was decreased 2 d and 6 weeks after kainate injection; whereas ATP levels were persistently decreased throughout the 6 week period. Evidence of CI modifications was measured throughout epileptogenesis. CI was immunoprecipiated from hippcomapal tissue and subunits were separated by SDS-PAGE. Ndusf1 modifications were identified with an Agilent 6510 QTOF LC/MS system and MS/MS data was analyzed with Mascot 2.2 (Matrix Science). In this study, we observed a time-dependent increase in Ndusf1 carbonylation throughout the course of epileptogenesis. This irreversible modification has been considered a biomarker for oxidative-induced cellular damage and may be a future therapeutic target in the prevention of epileptogenesis.

This work was supported by ROINS039587

PS 1298 TOLUENE EFFECTS ON OXIDATIVE STRESS IN BRAIN REGIONS OF YOUNG-ADULT, MIDDLE-AGE, AND SENESCENT BROWN NORWAY RATS.

P. S. Kodavanti, J. E. Royland, J. H. Richards, J. Besas and R. C. MacPhail. *NHEERL, U.S. EPA, Research Triangle Park, NC.*

Aging-related susceptibility to environmental chemicals is poorly understood. Oxidative stress (OS) appears to play an important role in susceptibility and disease in old age. The objectives of this study, therefore, were to test whether OS is a potential toxicity pathway for toluene exposure (a hazardous air pollutant) and to determine if these effects were age-dependent. We examined OS events to delineate reactive oxygen species production [NADPH Quinone oxidoreductase 1 (NQO1), NADH Ubiquinone reductase (UBIQ)], antioxidant homeostasis [total antioxidant substances (TAS) and superoxide dismutase (SOD)], and oxidative damage (total aconitase and protein carbonyls). Male Brown Norway rats (4, 12, and 24 months) were dosed orally with toluene (0, 0.65 or 1 g/kg) in corn oil. Frontal cortex (FC), cerebellum (Cb), striatum (Str), and hippocampus (Hip) were dissected 4 hours after exposure, quick frozen, and stored at 80°C until analysis. Results indicated constitutive age-related changes in some OS parameters in selected brain regions (eg NQO1 increase in Cb and TAS decrease in Str). Toluene effects on several OS endpoints were age- and brain region-specific. For example, toluene exposure increased NQO1 activity in FC and Cb at 4 and 12 months but only in Hip in 24 months. In contrast, toluene decreased TAS levels at 4 months in all brain regions and at 24 months in Cb, but increased TAS levels at 12 and 24 months in FC, Cb, and Hip. Markers of oxidative damage reached significance only at selected ages and/or doses. Aconitase levels were increased at 12 months in FC at 0.65 and 1.0 g/kg, 24 months in FC at 0.65 g/kg, and 24 months in Cb at 1.0 g/kg toluene. Significant increases in protein carbonyl levels in both FC and Cb matched the pattern of aconitase in the FC. These results indicate OS as a potential toxicity pathway, but the complex interaction between age and toluene exposure on OS parameters in different brain regions requires further investigation. (This abstract does not necessarily reflect USEPA policy).

PS 1299 MERCURY-INDUCED PHOSPHATIDIC ACID LIPID SIGNALING IN VASCULAR ENDOTHELIAL CELLS IS REDOX-REGULATED.

J. D. Secor[1, 2], R. B. Patel[1, 2], S. R. Kotha[1, 2], R. M. Uppu[3] and N. L. Parinandi[1, 2]. [1]*The Ohio State University College of Medicine, Columbus, OH,* [2]*Pulmonary, Dorothy M. Davis Heart and Lung Research Institute, Columbus, OH and* [3]*Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.*

Mercury has been implicated as a risk factor in cardiovascular diseases. However, the mechanisms of mercury-induced endothelial dysfunction are not known. Earlier, we reported that mercury induces activation of the lipid signaling enzyme, phospholipase D (PLD) in vascular endothelial cells. Here, we hypothesized that vascular endothelial cell PLD would be activated by mercury through the thiol-redox regulation. Hence, we used our well-established mouse aortic endothelial cell (MAEC) model and investigated the role of thiol-redox in the methylmercury (MeHg)-induced PLD activation. Our results demonstrated that: (i) MeHg induced PLD activation in dose-and time-dependent manner; (ii) thiol protectants such as N-acetyl-L-cysteine (NAC) and meso-2,3-dimercaptosuccinic acid (DMSA) attenuated MeHg-induced PLD activation; (iii) the novel, PLD-specific inhibitor, 5-fluoro-2-indolyl des-chlorohalopemide (FIPI), completely attenuated the MeHg-induced PLD activation; (iv) the novel thiol-protective compound, N,N'-bis(2-mercaptoethyl)isophtalamdie (BMEI), offered total attenuation of MeHg-induced PLD activation; (v) MeHg caused loss of cellular thiols and increase in ROS production in MAECs. Overall, our study demonstrated that MeHg-induced PLD activation was regulated by the thiol-redox alterations in vascular endothelial cells. Therefore, the PLD-mediated bioactive lipid signaling appears to play a role in the mercury-induced vascular disorders.

PS 1300 GENETIC ABLATION OF IPLA$_2$γ INCREASED URINARY MARKERS OF OXIDATIVE STRESS AND INCREASED LIPID PEROXIDATION IN KIDNEY CORTEX OF AGED MICE.

A. C. Eaddy, V. M. Kale, J. L. Blakely and R. G. Schnellmann. *Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.*

We previously showed that calcium-independent phospholipase A$_2$γ (iPLA$_2$γ), present in endoplasmic reticulum (ER) and mitochondria, protects renal cells from oxidant injury by preventing and/or repairing oxidant-induced lipid peroxidation. Oxidant stress is a major component of age-related deterioration of kidney function and structure, but the role of iPLA$_2$γ in this process has not been investigated. Thus, we utilized mice genetically ablated of iPLA$_2$γ (KO mice) to investigate its role in age-related oxidant injury and loss of kidney function. Urinary 8-isoprostanes, a biomarker of lipid peroxidation, and 8-hydroxy 2-deoxyguanosine (8-OH dG), a biomarker of oxidative DNA damage, were measured to assess oxidant injury. There were no differences in these markers in male KO and WT mice at 2 months of age. However, 13-month KO mice excreted 9-fold more 8-isoprostanes and 2-fold more 8-OH dG compared to 13-month WT mice. To determine kidney specific oxidant injury, we measured lipid peroxidation (TBARS) in mouse kidney cortex. There were no differences in lipid peroxidation in KO and WT kidney cortex at 2 months of age. However, kidney cortex of 13-month iPLA$_2$γ KO mice exhibited 1.7- fold higher TBARS content than 13-month WT controls. To assess kidney function, we measured serum and urine creatinine, urine volume, and urinary NGAL (a marker of acute kidney injury). There were no differences in these parameters between KO and WT mice at 2-months or 13-months of age. In summary, genetic ablation of iPLA$_2$γ increased excretion of 8-isoprostanes and 8-OH dG in the urine and increased lipid peroxidation in kidney cortex of male 13-month mice. However, at 13-months of age, the level of oxidant injury in the kidney did not result in renal failure. These data support our previous findings that iPLA$_2$γ protects renal cells from oxidative stress. This research was supported by F30 ES015964 and DK062028.

PS 1301 FREE FATTY ACIDS SENSITIZE HEPATOCYTES TO ETHANOL METABOLISM-INDUCED DAMAGE.

I. Hernandez Resendiz, L. Gomez Quiroz, L. Bucio, V. Souza and C. Gutierrez-Ruiz. *Health Sciences, Universidad Autónoma Metropolitana Iztapalapa, Mexico, Mexico.*

Palmitic (C16:0) and oleic (C18:1) acids are the predominant free fatty acids (FFA) in patients with NAFLD. Accumulation of FFA could induce reactive oxygen species (ROS) production.Hepatocytes have enzymatic, superoxide dismutas (SOD), caltalase (cat) and GSHpx, as and non-enzymatic systems, as gluthatione (GSH), that counteract oxidative stress. GSH is synthesized in two steps catalyzed by γglutamyl cysteine synthetase(γGCS) and GSH synthetase. γGCS is the rate limiting enzyme.Ethanol (EtOH) biotransformation by cytochrome 2E1 (CYP2E1) produces ROS. Delivery of free fatty acids to the liver may render hepatocytes more vulnerable to ethanol metabolism. Objective: to determine if FFA overload in the ethanol metabolizing cell line VL-17A enhance hepatocyte damage due to ethanol metabolism. The hepatic cell line VL-17A, obtained from HepG2 cells transfected with CYP2E1 and alcohol dehydrogenase, was used.Cells were seeded and cultured in presence of 1mM oleic and palmitic acids during 24 h. After that, cells were treated with 100 mM EtOH in presence of FFA for 48 h.Viability was determined by trypan blue assay.Intracellular lipids content were measured with red O oil.CYP2E1, cat, SOD 1 and 2,γGCS and GSHpx were determined by Western blot. VL-17A cells decreased viability 34% with FFA and 43% with FFA plus EtOH after 72 h treatment. Both treatments increased six times intracellular triglyceride content. Treatment with FFA and EtOH increased significantly SOD 1 and 2 and GSHpx compared with FFA treated cells, while no change in cat was found. γGCS decreased 50% and CYP2E1 increased significantly in FFA and EtOH treated cells. EtOH treatment in cells previously treated with FFA decreased viability; increased SOD1 and 2, CYP2E1 content and GSHpx and decreased γGCS. CYP2E1 rise could induce ROS production, and with the decreased cell capacity to produce GSH show that FFA treated cells are more susceptible to the harmful effects of ethanol metabolism.

PS 1302 ALCOHOL-INDUCED BONE LOSS IS BLOCKED IN P47PHOX -/- MICE LACKING FUNCTIONAL NADPH OXIDASES.

K. Mercer[2], R. Wynne[1, 2], C. Moutos[2], C. Lumpkin[1], L. Suva[1], T. Badger[1, 2], J. Chen[1, 2] and M. J. Ronis[1, 2]. [1]*University of Arkansas for Medical Sciences, Little Rock, AR and* [2]*Arkansas Children's Nutrition Center, Little Rock, AR.*

Chronic ethanol (EtOH) consumption produces bone loss. Previous data suggest a role for NADPH oxidase enzymes (Nox) since the pan-Nox inhibitor diphenylene iodonium (DPI) blocks EtOH-induced bone loss in rats. The current study utilized mice in which Nox enzymes 1,2,3 and 5 are inactivated as a result of knocking out the coactivator protein p47phox. C57BL/6 and p47phox -/-female mice (age 6 wk.) were pair-fed liquid diets of up to 30% EtOH for 4 wk.(blood EtOH 100-200 mg/d). In C57BL/6 mice, EtOH treatment reduced tibial cortical and trabecular bone density ($P<0.05$). EtOH-induced bone loss was completely prevented in p47phox -/-mice. Analysis of serum bone turnover markers revealed that EtOH treatment both increased bone resorption and decreased bone formation ($P< 0.05$). Resorption was unchanged in EtOH-treated p47phox -/- mice; whereas bone for-

mation was decreased to the same level observed in wild type mice. In vitro, EtOH treatment of bone marrow derived primary osteoblast (Ob) cultures from C57BL/6 mice which express Nox1, Nox2 and Nox4, resulted in induction of expression of receptor activator of NFκB ligand (RANKL) mRNA, an essential factor for osteoclast differentiation. However, no induction of RANKL was observed in cultures from p47phox -/- mice. Furthermore, in vitro studies with the pre-osteoblastic mouse ST-2 cell line demonstrated that EtOH-induced hydrogen peroxide production was inhibited by co-treatment with either 0.5 μM DPI or 5 μM of the Nox2 inhibitor gliotoxin. These data suggest that EtOH-induced bone loss is mediated at least in part by ROS generated via EtOH activation of Nox 1/2 enzymes in bone marrow Ob/pre-Ob and increased RANKL expression resulting in increased osteoclastogenesis and bone resorption. In addition, these data demonstrate that EtOH-inhibition of bone formation occurs via a Nox1/2-independent mechanism. Supported in part by R01 AA018282 (M.J.R.) and Carl L. Nelson Chair in Orthopaedic Creativity (L.S.).

PS 1303 INDUCTION OF OXIDATIVE DNA DAMAGE BY MESALAMINE IN THE PRESENCE OF COPPER: A POTENTIAL MECHANISM FOR MESALAMINE ANTICANCER ACTIVITY.

Z. Jia[1, 2], R. P. Zimmerman[1], H. Zhu[1], N. Vandjelovic[1], H. P. Misra[1], J. Wang[1] and Y. Li[1, 2]. *[1]Edward via Virginia College of Osteopathic Medicine, Virginia Tech Corporate Research Center, Blacksburg, VA and [2]Department of Biomedical Sciences & Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Mesalamine is the first line pharmacologic intervention for patients with ulcerative colitis, and recent epidemiologic studies have demonstrated a protective association between therapeutic use of the drug and colorectal carcinoma. However, the mechanism by which this protection is afforded has yet to be elucidated. Because copper is found at higher than normal concentrations in neoplastic cell nuclei and is known to interact with phenolic compounds to generate reactive oxygen species, we investigated whether the reaction of mesalamine/copper was able to induce oxidative DNA strand breaks in ϕX-174 RF I plasmid DNA, and the various components of the mechanism by which the reaction occurred. Plasmid DNA strand breaks were induced by pharmacologically relevant concentrations of mesalamine in the presence of a micromolar concentration of Cu(II), and damage was inhibited by bathocuproinedisulfonic acid (BCS) and catalase. Further, we showed that the reaction of copper with mesalamine consumed molecular oxygen, which was inhibited by BCS. Electron paramagnetic resonance spectral analysis of the reaction of copper/mesalamine indicated the presence of the hydroxyl radical, which was inhibited by both BCS and catalase. This study demonstrates for the first time that through a copper-redox cycling mechanism, the copper-mediated oxidation of mesalamine is a pro-oxidant interaction that generates hydroxyl radicals which may participate in oxidative DNA damage. These results demonstrate a potential mechanism of the anticancer effects of mesalamine in patients with ulcerative colitis.

PS 1304 EFFECT OF ZIDOVUDINE ON MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION PROTEIN LEVELS AND ENZYME COMPLEX ACTIVITIES.

J. Fang and F. A. Beland. *Division of Biochemical Toxicology, National Center for Toxicological Research/U.S. FDA, Jefferson, AR.*

The nucleoside reverse transcriptase inhibitor zidovudine (3'-azido-3'-deoxythymidine; AZT) is used extensively for the treatment of AIDS and the prevention of the mother-to-child transmission of human immunodeficiency virus type I. In HIV-infected patients exposed to AZT, clinical evidence of mitochondrial dysfunction, such as morphologically-damaged mitochondria, an increase in lactic acid production and changes in mtDNA quantity, has been observed. The present study examines the effects of AZT on mitochondrial membrane potential (ΔΨm) and the levels of oxidative phosphorylation (OXPHOS) complex subunits and their activity. Mouse fibroblasts NIH3T3 cells and human hepatoma HepG2 cells were incubated with AZT in continuous culture for up to 4 weeks. Using the lipophilic cationic fluorescent dye JC-1 as a probe, the ΔΨm was determined by flow cytometry. In NIH3T3 cells, AZT (0 – 1000 μM) treatment led to a significant dose-dependent loss in ΔΨm. In contrast, HepG2 cells exposed to AZT (0 – 100 μM) had a significant dose-dependent increase in ΔΨm. Western-blot analysis indicated no significant differences in the levels of OXPHOS complex subunits (39 kDa subunit of complex I, 70 kDa subunit of complex II, core 1 subunit of complex III, cytochrome c oxidase subunit II of complex IV, and ATP synthase α of complex V) or porin in the mitochondria isolated from AZT-treated NIH3T3 cells or HepG2 cells, or from liver, heart, and skeletal muscle of B6C3F1/TK+/- and B6C3F1/TK+/+ mouse pups exposed to AZT transplacentally from gestation day 12. The activities of OXPHOS complexes II, III, IV, and V were also evaluated and, again, there were no significant differences. These data indicated that AZT treatments that cause changes in ΔΨm do not result in the alterations in mitochondria function. (Supported by Interagency Agreement 224-07-0007 between NCTR/FDA and NIEHS/NTP.)

PS 1305 MECHANISMS BY WHICH GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE PROTECTS ITSELF AGAINST A CHEMICAL MODIFICATION.

T. Miura[1], Y. Egara[2], R. Hirose[1], N. Iwamoto[1], Y. Shinkai[1], A. K. Cho[3] and Y. Kumagai[1, 3]. *[1]Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan, [2]Master's Program in Environmental Sciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan and [3]Southern California Particle Center, University of California Los Angeles, Los Angeles, CA.* Sponsor: A. Naganuma.

Many cellular proteins with reactive thiols form covalent bonds with electrophiles that result in modification of structures and activities. Here we report that a glycolytic protein is able to recover from such covalent modification involving the electrophilic quinone, 1,2-naphthoquinone (1,2-NQ). We have found proteins that block the electrophilic attack by 1,2-NQ in the presence of NADH. Two dimensional SDS-PAGE and subsequent LC-MS/MS analysis revealed that a 37 kDa protein, identified as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was among them. This glycolytic enzyme readily underwent chemical modification by 1,2-NQ at a low concentration (0.2 μM) in a cell-free system, but exhibited minimal binding upon exposure to 1,2-NQ, even at 20 μM in human epithelial A549 cells while numerous other cellular proteins were extensively modified. In subsequent studies with purified human GAPDH, this enzyme was found to catalyze the reduction of 1,2-NQ to the non-electrophilic 1,2-dihydroxynaphthalene with NAD(P)H. Under these conditions, an NADH-dependent suppression of chemical modification of GAPDH by 1,2-NQ was observed. Although the adduct did form at high concentrations of 1,2-NQ, it underwent S-transalkylation with glutathione (GSH) to produce a GSH adduct of 1,2-NQ, which was associated with partial recovery of lost GAPDH activity. While GAPDH is well recognized to be a multifunctional protein, our results show that GAPDH also has a unique ability to repress its electrophilic modification by 1,2-NQ through 1) NAD(P)H-dependent two-electron reduction and 2) GSH-dependent S-transalkylation.

PS 1306 METABOLISM DEPENDENT MODULATION OF 3, 4-(±)-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED NEUROTOXICITY: POTENTIAL ROLE OF THIOETHER METABOLITES IN THE NEUROINFLAMMATORY RESPONSE.

J. M. Herndon, A. B. Cholanians, G. V. Erives, X. Li, S. S. Lau and T. J. Monks. *Pharmacology/Toxicology, University of Arizona - College of Pharmacy, Tucson, AZ.*

3,4-(±)-Methylenedioxymethamphetamine (MDMA) abuse is a significant problem in the United States and around the world. Despite the widespread use of MDMA, relatively little is known about the underlying mechanisms of MDMA-induced neurotoxicity. We now describe studies on the role of metabolism in MDMA-induced neurotoxicity, as well as the potential role of thioether metabolites of MDMA in the neuroinflammatory response. Cytochrome P450 family member 2D6 (CYP2D6) and catechol-O-methyl transferase (COMT) participate in the metabolism of MDMA. These enzymes were inhibited in either pharmacological or genetic models to assess the effects of this inhibition on MDMA-induced neurotoxicity. Animals were administered a neurotoxic dose of MDMA (20 mg/kg sc - rats, 30 mg/kg sc x 3 at 3 hour intervals - mice) and neurotransmitter levels were determined by HPLC coupled to Electrochemical Detection, as a marker of neurotoxicity. For inflammatory studies, microglial activation was used as an indirect measure of inflammatory status in the CNS. Both IHC and a functional fluorescence assay were used to determine microglial activation. Inhibition of CYP 2D6 caused a marked attenuation of MDMA-induced neurotoxicity, whereas inhibition of COMT resulted in a potentiation of MDMA-induced neurotoxicity. Preliminary data indicates that peripheral administration of MDMA or direct intracranial injection of thioether metabolites of MDMA causes an increase in microglial activation 72 hours following administration. Perturbations in the enzymes that participate in the metabolism of MDMA result in a change in the neurotoxic profile presumably due to a change in the amount of thioether metabolites formed. When thioether metabolites are injected directly into the brain, an increase in microglial activation is observed, which could contribute to an inflammatory cascade in the CNS which exacerbates MDMA-induced neurotoxicity.

PS 1307 PRO-OXIDANT ACTIONS OF APOCYNIN AND ITS OXIDATIVE METABOLITES DIAPOCYNIN AND 5-NITROAPOCYNIN.

L. A. Laynes[1], A. C. Raghavamenon[1] and R. M. Uppu[1, 2]. [1]*Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA and* [2]*Chemistry, Southern University and A&M College, Baton Rouge, LA.*

Apocynin (Apo), a methoxy catechol isolated from the Indian hemp (*Apocynum cannabinum*) is known for its antioxidant and inflammatory properties. The present study investigated the pro-oxidant actions of apocyanin (Apo) and its two major oxidative metabolites, diapocynin (DiApo) and 5-nitroapocynin (5-NitroApo). The phenolate radicals of Apo, DiApo and 5-NitroApo generated by the oxidation of the corresponding methoxy catechol, catalyzed by horseradish peroxidase (HRP)-H_2O_2 system, were allowed to react with GSH and NADPH. It was found that radicals of Apo and 5-NitroApo, but not DiApo, significantly enhanced the rate of oxidation of GSH and NADPH. Trolox, which was used as a positive control, inhibited the oxidation of both GSH and NADPH. Apo and 5-NitroApo were found to result in a 3.1 (± 0.59, mean ± SD, n = 3)- and 8.2 (± 2.14, mean ± SD, n = 3)-fold increase, respectively in the rate of Trolox oxidation induced by HRP-H_2O_2. DiApo, under the identical conditions, apparently had little or no effect on the oxidation of Trolox. DiApo showed a greater efficacy in scavenging the 1-diphenyl-2-picrylhydrazyl radical (DPPH; 21.5 ± 2.33, mean ± SD, n = 3) as compared to that exhibited by Apo (6.7 ± 1.27, mean ± SD, n=3) and 5-NitroApo (7.5 ± 0.62, mean ± SD, n = 3), indicating a free-radical scavenging action of DiApo as opposed to the pro-oxidant actions of Apo and 5-NitroApo. Over all, the results of the present study revealed that 5-NitroApo exhibited pro-oxidant actions which were greater in magnitude than those exhibited by the parent molecule, Apo and also that DiApo was more likely to meet the criteria as an antioxidant *in vivo*. [Funding support from NIH (P20 RR16456) and US Department of Education (PO31B040030) is acknowledged. Corresponding author's email: rao_uppu@subr.edu].

PS 1308 GLUTATHIONE AND AMINO ACID FLUX MEASUREMENT UNDER NORMAL AND OXIDATIVE STRESS CONDITIONS USING HPLC AND ACCELERATOR MASS SPECTROMETRY.

B. Stewart[1], A. Navid[2], R. Le[1], K. Turteltaub[2] and G. Bench[1]. [1]*Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA and* [2]*Bioscience and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA.*

The ability to accurately measure metabolic fluxes in cells and tissues can be used to enhance our understanding of biological responses to oxidative stress. We performed experiments in the model organism Saccharomyces cerevisiae to evaluate the effects of mild to moderate oxidative stress on amino acid uptake and metabolic flux of glutamine proceeding to glutathione biosynthesis. Yeast cells growing in log-phase were labeled with 14C-glutamine, and exposed to 100 μM hydrogen peroxide or were untreated. Extracellular intracellular amino acids were measured over a time course by high-performance liquid chromatography (HPLC). Metabolic fluxes from glutamine through glutamate and glutathione were measured by 14C detection by accelerator mass spectrometry. Results show that a mild oxidative stress initially impaired cellular uptake of amino acids, and subsequently accelerated flux of 14C label from glutamine to GSH over fluxes measured in control cells. Measurable metabolic effects were observed up to 135 minutes post-exposure, followed by metabolic re-equilibration. These experiments demonstrate the utility of a targeted labeling approach for measurement of low-level perturbations of metabolic fluxes using nCi (pmol) levels of 14C-labeled tracer, with sensitivity at the attomole level.

R 1309 INTEGRATING ALTERNATIVE TEST METHODS INTO THE FEDERAL REGULATORY FRAMEWORK.

S. C. Fitzpatrick[1] and L. Schechtman[2]. [1]*Office of the Commissioner, U.S. FDA, Silver Spring, MD and* [2]*Innovative Consulting, LLC, Lake Worth, FL.*

Current regulatory testing paradigms require certain sets of data needed for regulatory evaluation and risk assessment. Many of the methodologies used today in the traditional approach to toxicity testing originated over a half century ago and are both time and animal intensive. In 2007, the National Academy of Sciences report described a new vision and strategy for toxicity testing in the 21st century that would be based on human biology rather than animal biology and would be less expensive and less time consuming. This strategy would also involve a strong commitment to the 3Rs—replacement, reduction, and refinement of animal use in research, testing, and education. Being responsive to this progressive scientific perspective would necessitate moving forward in developing, validating, and incorporating alternative toxicological test methods into the federal regulatory framework. This transformational toxicological approach would also entail an active dialogue and collaboration between stakeholders, NGOs, academics, and federal scientists. Our panel of experts will offer the opportunity for such a discourse between U.S. FDA and U.S. EPA and their stakeholders about the type of data needed from alternative methods to demonstrate that they are scientifically valid and address the fundamental questions of human safety and efficacy.

IS 1310 COORDINATING GLOBAL CHEMICAL SAFETY: THE BIG FOUR.

P. Wexler[1], P. Whiting[2], D. Downie[3], H. Selin[4] and L. Onyon[5]. [1]*National Library of Medicine, Bethesda, MD,* [2]*U.S. EPA, Washington, DC,* [3]*Fairfield University, Fairfield, CT,* [4]*Boston University, Boston, MA and* [5]*UNEP, Geneva, Switzerland.*

As the toxicology community is well aware, chemicals are both a global benefit and concern. They routinely cross national borders in the marketplace and in planned or accidental pollution streams. With increasing production comes increasing opportunity for exposure. Novel chemical entities, such as those derived from nanotechnology, simply up the ante with still undetermined effects. It has been nearly 40 years since the United Nations (Stockholm) Conference on the Human Environment stated in its declaration that 'The protection and improvement of the human environment is a major issue which affects the well-being of peoples and economic development throughout the world; it is the urgent desire of the peoples of the whole world and the duty of all Governments.' Principle 6 of this declaration also indirectly advocated control of chemicals by calling for a halt to the discharge of toxic substances which exceed the capacity of the environment to render them harmless. The strengthened global will to manage chemicals responsibly has been facilitated by advances in information and communications technologies, chief among them being the Internet. Many multilateral efforts, both legally binding vehicles and policy frameworks, can at least, in some respect, trace their lineage back to the Stockholm Conference. These three major international legal instruments dealing with chemicals including the transboundary movement of hazardous wastes and their disposal, prior informed consent, and persistent organic pollutants will be our focus. We will highlight the Strategic Approach to International Chemicals Management (SAICM), today's major international policy framework. Finally, our panel of experts will offer a summary relative to the provisions of these multilateral environmental agreements (MEAs), highlight major successes and challenges, discuss future outlooks, and indicate the relevance of global chemical policy to toxicologists, and how they, in turn, can influence such policy.

IS 1311 LIVERS ON A PLATE: NEXT GENERATION HEPATOCYTE MODELS FOR HIGH-THROUGHPUT SCREENING AND MODE-OF-ACTION PREDICTION.

C. Corton[1], S. Khetani[4], L. Griffith[3], D. Applegate[2] and S. D. Hester[1]. [1]*Integrated Systems Toxicology Division, U.S. EPA, Durham, NC,* [2]*RegeneMed, Inc., San Diego, CA,* [3]*MIT, Cambridge, MA and* [4]*HepreGen Corporation, Medford, MA.*

The liver is a common target for chemicals and drugs, due in part to the role of the liver in xenobiotic metabolism. Because the liver is frequently the most sensitive target in two-year bioassays, chemical regulation is often based on adverse liver effects. Drugs withdrawn from the market are more often due to unforeseen hepatotoxicity. Given the number of chemicals that need to be assessed for hepatotoxicity by the pharmaceutical and chemical industries, many investigators currently use primary hepatocytes or transformed hepatocyte-derived cell lines in lieu of animal studies. These models have serious limitations including major differences in the expression of xenobiotic metabolizing enzymes compared to the intact liver. There is a clear need for the identification and characterization of biologically-relevant in vitro models that can recapitulate the functions, cell interactions, and responses to chemicals found in vivo. A number of exciting in vitro models of the liver have been recently developed which make them potentially better models for predicting responses in vivo than conventional cultures. We will characterize the use of a number of in vitro models of the liver including 2D and 3D co-cultures containing hepatocytes and other cell types. Our panel of experts have an extensive experience in the characterization and use of these models and will discuss applications of the models including toxicity screening, metabolic activation, mode-of-action determination using toxicogenomics and species extrapolation. This particular topic will be useful to those interested in high-throughput screening, hepatotoxicity, and mode-of-action research.

PS 1312 EVALUATION OF THE SENSITIVITY OF ENDPOINTS IN THE ISO 10993-11 SYSTEMIC TOXICITY STANDARD USING POSITIVE CONTROL COMPOUNDS.

A. Freeman[1, 2], A. Komiyama[1], J. Fazio[1, 3], S. Hamilla[1, 4], H. Dinesdurage[1] and R. P. Brown[1]. *[1]U.S. FDA, Silver Spring, MD, [2]University of Maryland, College Park, MD, [3]Western New England College, Springfield, MA and [4]University of Connecticut, Storrs, CT.*

The preclinical biological safety evaluation of medical devices often includes an assessment of acute systemic toxicity. This assay is typically conducted using the test method outlined in the ISO 10993-11 (2008) standard. Criteria are included in the standard to determine if an extract of a device is toxic or not. These criteria include death of one or more animals, behavioral changes, or greater than 10% change in body weight (BW). Since the test methods outlined in this standard have been criticized as being too insensitive to detect toxic effects, a proof-of-principle study was conducted to compare the sensitivity of these endpoints to clinical chemistry results for two positive control compounds, cadmium chloride (Cd) and mercuric chloride (Hg). Intraperitoneal injection of the compounds (0.5 or 1 mg/kg) to male CD-1 and Balb/c mice resulted in slight change in body weight after 24 hours, compared to saline injected controls; however the changes were less than 10% of the total BW. There was no statistically significant difference in BW between treated and control animals at the 72-hour time point. Behavioral changes were observed in high-dose Cd animals shortly after injection, but not those treated with 0.5 mg/kg; however, ALT levels were elevated, relative to controls, at both doses, but only at the 24-hr time point. These results suggest that the endpoints in the standard are sufficiently sensitive to detect overt but not subtle signs of toxicity. The incorporation of endpoints such as clinical chemistry may improve the sensitivity of the acute systemic toxicity assay for some compounds.

PS 1313 RAPID TOXICITY SCREENING OF POLYMERIC MATERIALS USING THE MICROTOX ASSAY.

P. Kulkarni, H. Dinesdurage and R. P. Brown. *CDRH, U.S. FDA, Silver Spring, MD.*

Cytotoxicity is assessed as part of the biological safety evaluation of essentially every new medical device. The *in vitro* cytotoxic effects of device extracts are typically assessed using cells in suspension (e.g., direct hemolysis) or in culture. The Microtox assay, based on light emission from photoluminescent bacteria (*Vibrio fischeri*), is widely used in aquatic toxicology to screen water samples for cytotoxic effects. The potential for these bacteria to be used as an adjunct to the use of mammalian cell-based cytotoxicity assays for rapid toxicity screening of extracts of device materials was evaluated. In this study, the direct hemolysis assay was conducted using a modification of the method in the ASTM F756 standard to classify polymeric materials (BUNA, nitrile, latex (2 types), neoprene, silicone, polyurethane, high density polyethylene, butyl rubber, norprene, polypropylene, F-4040-A tubing) as being toxic (≥5% hemolysis) or nontoxic. The materials were then extracted in 50% ethanol (24 hrs at 37°C) and the extracts were used in the Microtox and indirect hemolysis assays. Microtox was able to differentiate toxic from nontoxic materials with a sensitivity of 100% and a specificity of 57%, assuming cell death ≥ 10% represents a toxic response in this assay. There were no false negatives and three false positives, suggesting that the Microtox assay is more sensitive than the direct hemolysis assay. There was complete agreement between the results of the Microtox assay and the indirect hemolysis assay. Since the Microtox assay is sensitive, does not require sterile technique and results can be obtained in 15 minutes, it represents a promising adjunct to the direct and indirect hemolysis assays for the rapid toxicity screening of polymeric materials used to manufacture medical devices.

PS 1314 USE OF AN *IN VITRO* SCREEN FOR ASSESSING THE VAGINAL IRRITATION POTENTIAL OF INTIMATE CARE PRODUCTS.

P. Clay[1] and N. Belot[2]. *[1]SSL International, Manchester, United Kingdom and [2]Straticell, Gembloux, Belgium.* Sponsor: D. Kent.

Intimate care products such as personal lubricants and vaginal moisturisers are classified as medical devices in certain regulatory regions. When developing new products, assessment of the irritancy of the product forms part of the assessment of the overall biological safety prior to performance evaluation in volunteers. If considered necessary, the pre-clinical assessment of irritation potential can include a vaginal irritation study in the rabbit (ISO 10993/10). A recent product development called into question the sensitivity and relevance of this test to the effects seen in humans for this type of product and therefore an alternative/additional test was sought. For ethical reasons, an *in vitro* test method has been developed; this also offers potential cost and time benefits which may make the method suitable as an early candidate screen. The test methods used were those given by SkinEthic Laboratories for use with their Skin Ethic HVE™ reconstituted human vaginal epithelium model. Briefly, day 6 cultures of tissue were treated in duplicate with 70µL of the test sample for 20mins, 1, 2 and 4 hours after which the viability of the tissue was assessed by MTT assay. From these data, an ET50 (time taken to reduce viability to 50% of the control) value was calculated and used as an indictor of the irritant potential of the formulation. Initial experiments with a range of commercially available vaginal moisturisers (Replens MD, Saugella Intilac, Monasens Intimate Care, Durex Sensilube) suggested that these methods could provide an appropriate level of sensitivity and also showed a good correlation with irritancy reported in volunteer trials. Further validation of the methods to establish inter-experimental variability has shown the methods to be highly reproducible and confirmed the sensitivity of the methods. These experiments demonstrate that the methods used are suitable as a screen for candidate formulations and have the potential to offer more relevant sensitivity than the current animal tests used for regulatory biocompatibility assessment.

PS 1315 DETERMINATION OF BISPHENOL-A COMPOUNDS LEACHABLE FROM POLYCARBONATE- AND POLYSULFONE-BASED HEMODIALYZERS.

J. Guo, S. Cho and H. D. Luu. *Division of Chemistry and Materials Sciences, U.S. FDA, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Silver Spring, MD.*

Bisphenol A (BPA) is an organic compound used to produce polycarbonate (PC) and polysulfone (PS) plastics. Both polymers have been widely used, not only for consumer products such as baby and water bottles and electronics, but also for medical devices. However, many studies have reported that BPA acts as an endocrine disruptor, which is a possible health hazard to humans, especially to the pediatric population. Recent concerns over the release of BPA from PC- and PS-based products in daily use have prompted us to measure the amount of BPA compounds leachable from PC- and PS-based medical devices. In particular, hemodialyzers have a high possibility of exposing residual or hydrolytically generated BPA to patients, because these devices have direct contact with the blood stream during extracorporeal circulation. In this study, we focused on identifying and quantifying BPA compounds leachable from PS/PC-based hemodialyzers. We used exhaustive extraction methods to determine the maximum BPA present as a worst case scenario and dynamic extraction methods to determine the amount of BPA leachable under simulated physiological conditions. Finally, we used gas chromatography/mass spectrometry (GC/MS) to analyze the BPA compounds.

PS 1316 HUMAN BIOKINETIC MODEL OF NICKEL RELEASE FROM MEDICAL DEVICES.

J. S. Tsuji[1] and K. T. Bogen[2]. *[1]Exponent, Bellevue, WA and [2]Exponent, Oakland, CA.*

Quantifying the magnitude of nickel (Ni) release over time is important for understanding the potential for health impacts of medical devices containing Ni. Transient increases in Ni serum levels have been reported for patients with implanted medical devices made of Ni-containing alloys (e.g., stainless steel, nitinol). Nitinol (55% titanium/45% Ni) has been used in metal wire structures of various cardiovascular occlusion devices, such as for closure of atrial septal defects or as a vascular plug. Ni release rates and their timing in vivo, however, cannot be directly inferred from discrete measurements of Ni serum concentrations because of the complex biokinetics of Ni in the body, affecting both the magnitude of serum levels and their time course. A biokinetic model for internal Ni release was developed using a published short-term model based on oral dosing of Ni in humans, as modified to include more complicated and longer-term kinetics noted in other studies. The model was fit to Ni serum data at discrete time points reported in clinical studies of patients with implanted nitinol devices (septal occluders), with adjustment of parameters as necessarily and derivation of corresponding Ni release rate curves. The overall pattern of Ni release predicted by the model showed increases in the Ni release rate after implantation that peaked within the first week. The predicted in vivo release rates declined to zero by 145 days after implantation. Estimated total Ni released per patient was less than 1/10th of the normal human Ni body burden, and represents a relatively small amount of Ni dosing to the body at any one time. By comparison, internal Ni releases occur over much longer time periods for other medical devices such as stainless steel spinal implants based on elevated serum Ni levels up to 4 years post-implantation. Ni-containing medical devices in humans or dogs, however, have not been associated with long-term health effects due to high Ni release, as observed in rodents injected or implanted with pure Ni powders or pellets.

PS 1317 PRECLINICAL SAFETY ASSESSMENT OF DESMA (CASRN 1101874-33-2) FOR USE IN PROLONGED SURFACE CONTACT DENTAL PRODUCTS.

B. D. Bagley[1], L. H. Moilanen[1] and K. Stefan[2]. [1]*Medical Department, 3M, St. Paul, MN and* [2]*3M ESPE, Seefeld, Germany.*

DESMA (CASRN 1101874-33-2) is a complex polyurethane methacrylate mixture developed and manufactured by 3M ESPE (Seefeld, Germany) for use as a major monomer in temporary crowns and bridges. DESMA was evaluated prior to use in products for cytotoxicity, sensitization, skin and ocular irritation, acute oral toxicity, and genotoxicity in accordance with recommendations in ISO Standards 10993 and 7405. DESMA was not cytotoxic in a XTT test performed using mouse L929 cells according to ISO 10993-5: 1999. DESMA showed no evidence of sensitization in a mouse local lymph node assay performed according to OECD Guideline 429 (2002). DESMA was not a skin irritant in a primary skin irritation test performed according to OECD Guideline 404 (2002) in New Zealand White rabbits. DESMA did not show potential for eye irritation in a bovine corneal opacity and permeability (BCOP) assay performed according to INVITTOX protocol number 98 (1994). The rat acute oral LD_{50} of DESMA is >2000 mg/kg based on a test performed according to OECD Guideline 423 (2001) in Wistar rats. DESMA was not mutagenic at up to 5000 µg/plate in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or *E. coli* strain WP2*uvr*A in either the presence or absence of metabolic activation in assays performed according to OECD Guideline 471 (1998). DESMA was not mutagenic in cultured L5178Y/TK +/- cells in a mouse lymphoma assay performed according to OECD 478 (1998). These test results confirm that DESMA is safe for its intended use as a major component of dental devices designed for prolonged surface contact.

PS 1318 REPRODUCTIVE TOXICITY EVALUATION OF THE RESIN MONOMER TRIETHYLENE GLYCOL DIMETHACRYLATE (TEGDMA) IN MICE.

L. H. Moilanen[1], J. K. Dahms[3] and A. Hoberman[2]. [1]*Medical Department, 3M, St. Paul, MN,* [2]*Charles River Laboratories, Horsham, PA and* [3]*3M ESPE, St. Paul, MN.*

Triethylene glycol dimethacrylate (CASRN 109-16-0; TEGDMA) is widely used in dentistry as a monomer in composite restorative materials, where it functions as a low viscosity diluent and crosslinking agent. Residual TEGDMA monomer may leach from polymerized composite material into the oral cavity, resulting in systemic exposure. Two recently published studies report possible reproductive effects in mice following oral exposure to TEGDMA, but interpretation of the results is constrained by lack of test article characterization and use of a non-standard study design. To clarify possible adverse effects, the reproductive toxicity potential of TEGDMA was investigated in male and female Crl:CD1(ICR) mice (25 mice/sex/group) in a definitive GLP-compliant study conducted according to recommendations in OECD 415. TEGDMA (0.01, 0.1 or 1.0 mg/kg-day) in deionized water was intubated once daily beginning 28 days before cohabitation and continuing through mating (males) or through gestation day 17 (females). Parameters evaluated included viability, clinical signs, body weights, estrous cyclicity, necropsy observations, organ weights, sperm concentration/motility/morphology, Caesarean-sectioning and litter observations and histopathological evaluation of select tissues. No deaths or clinical signs related to TEGDMA occurred. No significant changes in male and female body weights or body weight gains occurred with any administered dosage of TEGDMA. All mating and fertility parameters, and all litter and fetal data, were unaffected by treatment at dosages up to 1 mg/kg-day. No gross or histopathologic tissue changes attributable to the test article were observed. The reproductive and developmental NOAELs for TEGDMA were thus 1 mg/kg-day, the highest dose tested. The results of this study indicate low potential for male or female reproductive effects associated with the use of TEGDMA in dental restoratives at levels of exposure consistent with dosages employed in this study.

PS 1319 PRECLINICAL BIOCOMPATIBILITY ASSESSMENT OF A NEW NANO-FILLED FLOWABLE DENTAL COMPOSITE.

E. F. Hope[1], L. H. Moilanen[1] and A. Bogdan[2]. [1]*Medical Department, 3M, St. Paul, MN and* [2]*3M ESPE, St. Paul, MN.*

3M™ ESPE™ Filtek™ Supreme Ultra Flowable Restorative is a new nano-filled, visible light-activated flowable composite designed for use in anterior and posterior dental restorations. Based on the innovative nature of this product, a comprehensive preclinical biocompatibility assessment of the product was required prior to application in patients. The assessment was conducted within a risk management framework as recommended in ISO 10993-1:2009, and included material characterization and biocompatibility testing components. The product was initially characterized by extraction of cured discs in each of five solvents according to general guidelines in ISO 10993-12, followed by gravimetric and HPLC analysis of the extracts. In the second stage of the assessment, biocompatibility tests were selected in accordance with recommendations for an externally communicating product in permanent (>30 days) contact with tissue, bone or dentin, and included cytotoxicity, intracutaneous reactivity, sensitization, acute toxicity, repeated-dose toxicity, genotoxicity, and implantation. Material characterization studies indicate total leaching in six weeks of less than 0.1% in a clinically relevant solvent, and 3.2% or less in exaggerated extractions using methanol or acetone. A culture-medium extract of cured discs of the product was cytoxic (IC50 = 39.02% v/v) in a Colony Forming Ability cytotoxicity assay. Subsequent definitive GLP- and ISO 10993-compliant *in vivo* studies indicate that polar and non-polar extracts of cured discs of the product were not irritating, did not induce sensitization, were not acutely toxic (LD50> 10 mL extract/kg), and were not genotoxic in bacteria or cultured mammalian cells. No adverse effects were observed in rats orally dosed with aqueous product extracts for 28 days (NOAEL = 52 µg extract residue/kg-day). No adverse local tissue effects were observed when cured discs were implanted in implanted in muscle tissue for up to 90 days. The results of this assessment confirm the safety of this product when used as intended for dental restorations.

PS 1320 POLYPHENOL-ELUTING STENT COATING, A NOVEL APPROACH FOR THE PREVENTION OF RESTENOSIS WITH REDUCED SIDE EFFECTS.

J. J. Kleinedler[1], A. W. Orr[2], V. Hebert[1], A. Yurdagul[2], J. D. Foley[3] and T. R. Dugas[1]. [1]*Pharmacology, Toxicology, and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, LA,* [2]*Pathology, Louisiana State University Health Sciences Center, Shreveport, LA and* [3]*Biomaterials Research, Nanocopoeia, Inc., St. Paul, MN.*

Restenosis, an adverse outcome of angioplasty, is characterized by thickening of the arterial wall and re-narrowing of the arterial lumen. Current generation coronary drug-eluting stents (DES) used to treat restenosis are associated with unintended complications including potentially fatal late-term thrombosis. Several studies indicate that unfavorable outcomes after DES placement may be due to toxicity of the active compounds to endothelial cells, leading to delayed vascular healing. This provides an impetus for the development of novel drug-eluting coatings that reduce restenosis with reduced toxicity to the endothelium as to promote vascular healing. Due to the vascular-protective effects of red wine, we chose to investigate whether two of the most potent compounds derived from wine, resveratrol (RESV) and quercetin (QUER), would interfere with multiple steps in the pathogenesis of restenosis and thrombosis. In vitro isobolographic analysis identified synergistic combinations of RESV and QUER that inhibited proliferation and activation of vascular smooth muscle cells and macrophages, respectively. Since the risk of late thrombosis is primarily attributed to delayed re-endothelialization, we studied the effect of RESV on endothelial cell (HAEC) proliferation and migration under static or laminar shear stress (LSS) conditions. We found that RESV promoted HAEC migration under LSS at the same concentration that inhibited smooth muscle cell migration. This suggests that RESV may promote vascular healing while inhibiting restenosis. To test this hypothesis in vivo, we assessed the efficacy of miniaturized stents coated with RESV and QUER to inhibit restenosis. We found that the local delivery of RESV and QUER from a stent platform significantly reduced restenosis compared to bare metal stents. This data suggests that RESV and QUER may have utility as therapeutics with reduced toxicity in a novel coating for DES.

PS 1321 CYTOTOXICITY TESTING STRATEGY OF CONTACT LENS DISINFECTING SOLUTIONS.

S. Kostrubsky, K. Bullard and J. Wegrzyn. *Vistakon, Division of J&J Vision Care, Jacksonville, FL.*

In Vitro testing of multi-purpose solutions represents a challenge since any ingredient of a multi-component mixture can contribute to the toxic effect. The colony assay using V79 cells, is a sensitive cytotoxicity test conducted on cells in the growing phase either by direct contact of the device with plated cells or via cells exposed to an extract of a device prepared in culture media. Another test is the direct contact using a monolayer of sub-confluent L929 cells exposed to a device for 24 hr representing a less sensitive cytotoxicity assay. Using all three methods, we studied cytotoxicity of different solutions with and without exposure of contact lenses. Lenses were thoroughly rinsed with saline prior to being placed in the test. Thus, only effect of chemical ingredients adsorbed by the lens material and released into the media was investigated resembling chronic exposure. The following marketed solutions were studied: Menicare soft, Puracl, Complete RevitaLens (CRL), BioTrue, ReNu MultiPlus and Opti-Free Replenish (OFR). All solutions are clinically safe. However, in vitro methods allow ranking of these solutions according to cytotoxic potential. We found 100% inhibition of colony growth by lenses exposed to undi-

luted OFR and CRL. Culture media extracts also were prepared from lenses exposed to solutions and rinsed with saline prior to extraction. No colony growth was observed in cells treated with extracts of OFR- and CRL-treated lenses. There was no toxicity in any untreated lenses. Direct contact test conducted in L929 cells under the same conditions reveled a slight zone of cell lysis only under the lenses soaked in OFR and CRL. Importantly, exposing cells directly to solutions or unrinsed lenses produces different results due to contributions of surfactants and buffering agents similar to acute ocular exposure. In summary, as a part of product development, we propose this strategy to identify solution with less potential for toxicity resulting from exposure to either complete formulation or released ingredients from lenses.

PS 1322 TRANSLATIONAL IMAGING OF AN INJECTABLE POLY-L-LACTIC ACID DERMAL FILLER.

N. J. Barlow[1], D. Wilson[1], R. Rimsky[1], L. Wachsmuth[1], T. Coulthard[2] and X. Ying[1]. [1]*Disposition, Safety and Animal Research, sanofi-aventis U.S., Inc., Bridgewater, NJ and* [2]*VisualSonics, Inc., Toronto, ON, Canada.*

Sculptra® Aesthetic is a dermal filler composed of injectable poly-L-lactic acid (PLLA) that is approved for the treatment of mild to deep nasolabial fold wrinkles. The purpose of this translational imaging study was to determine the visibility of the PLLA by various clinical imaging technologies, including computed tomography (CT), radiography (X-ray), magnetic resonance imaging (MRI), and ultrasound imaging. Rats received Sculptra® Aesthetic by subcutaneous injection at two distinct sites on Days 1 and 90. Rats were imaged on Day 91 and skin from the two injection sites and an untreated site was collected for a correlative microscopic examination. The PLLA was well visualized by ultrasound 24 hours after injection; but, was poorly visualized by ultrasound 90 days after injection and the observations may not have been due to the PLLA because there was a lack of microscopic correlation with the ultrasound findings. Sculptra® Aesthetic was well visualized with MRI 24 hours after injection in T1, T2 and/or T2*-weighted images. However, after 90 days the appearance of the injection site did not differ from the untreated reference site using MRI. Sculptra® Aesthetic was not visualized with CT and radiography at either time point. Microscopically, Sculptra® Aesthetic was observed 24 hours after injection in the subcutaneous tissue and was associated with acute inflammation; these findings correlated with the observations visualized by ultrasound and MRI imaging. The PLLA was also observed microscopically in the subcutaneous tissue 90 days after injection and was associated with granulomatous inflammation. The respective inflammation at 24 hours and 90 days was an expected acute and chronic foreign body reaction to the PLLA. In summary, the PLLA-based dermal filler Sculptra® Aesthetic was visualized in rat skin 24 hours following subcutaneous injection using ultrasound and MRI; however, it was not definitively observed with the various imaging modalities after 90 days.

PS 1323 EVALUATION OF EXTRACTABLES/LEACHABLES FROM IMPLANTED MEDICAL DEVICES.

L. A. Haighton[1, 2], H. Fikree[2], L. Sarkissian[2] and J. W. Card[2]. [1]*Cantox Health Sciences International, Mississauga, ON, Canada and* [2]*Ashuren Health Sciences, Mississauga, ON, Canada.*

Safety evaluation of implanted medical devices has traditionally focused on biocompatibility issues (tissue reactivity, inflammation, immunogenicity, cytotoxicity); however, consideration of potential leachables is now a necessary component. Even though leachables typically arise in only trace amounts, the assessment of leachables can add greatly to the cost of developing medical devices with the expected financial repercussions to the patient. An approach is proposed that combines existing knowledge of risk assessment methods and extractables/leachables (E/L) assessment practices from inhalation devices to derive thresholds to support the safety of implanted medical devices with limited need for additional toxicology testing. This approach takes into consideration the differences in exposure routes and the fact that E/L have varying degrees of diffusion and hydrophilicity which factor into the potential exposure duration. Highly hydrophilic residuals are likely to be leached (and exhausted) early from medical devices and would have a short residence time; conversely, very hydrophobic compounds may be leached more gradually from the medical device, resulting in a longer exposure period. Thus, the principle concepts in deriving thresholds specific for E/L from an implanted medical device are: (i) the justification for the use of thresholds in risk assessment; (ii) the importance of exposure duration in determining risk and the science supporting that exposure to low doses over an extended period of time is more potent than higher-dose exposure for a shorter duration; (iii) the potential for organ toxicity which is dependent upon the amount of leachable systemically available (bioavailability differences between inhalation and implantation); and, (iv) target patient population. The value of this approach is demonstrated by applying the derived thresholds to representative E/L with varying exposure profiles.

PS 1324 AGE-RELATED DIFFERENCES IN CHOLINESTERASE (CHE) INHIBITION IN IMMATURE AND ADULT RATS AFTER ACUTE OR REPEATED EXPOSURES TO CHLORPYRIFOS (CPF) OR CPF-OXON (CPFO).

M. S. Marty[1], A. K. Andrus[1], M. P. Bell[1], J. K. Passage[1], A. W. Perala[1], K. A. Brzak[1], S. N. Fishman[1], M. J. Bartels[1] and D. R. Juberg[2]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Dow AgroSciences, Indianapolis, IN.*

To examine age-related differences in ChE inhibition, PND 11 rats (8/sex/dose) and adult female rats (8/dose) were exposed acutely or repeatedly to CPF or CPFO by gavage in corn oil. Samples were collected at time-of-peak ChE inhibition and analyzed via modified Ellman method. Blood levels of CPF, CPFO, and/or trichlorpyridinol were determined. Acute CPF studies: ChE activity was measured after a single dose of 0, 0.05, 0.1, 0.5, 2, 5 (pups only) or 10 (adults only) mg/kg CPF. Both pups and adults had comparable decreases in brain ChE at the highest CPF dose (5 mg/kg in pups; 10 mg/kg in adults) and significant RBC ChE inhibition at ≥2 mg/kg, although pups had higher plasma CPF levels at each dose level. In both age groups, ChE inhibition $NOEL_{brain}$ = 2 mg/kg and $NOEL_{RBC}$ = 0.5 mg/kg CPF with acute exposure. Repeated CPF studies: ChE was measured in PND 21 pups and adult females after 11 daily exposures to 0, 0.05, 0.1, 0.5, 1, 3.5 mg/kg/day CPF. At 3.5 mg/kg/day, pups had similar ChE inhibition to adults across all tissues, despite having higher plasma CPF levels. At 0.5 mg/kg/day, RBC ChE was inhibited in adults and pups. In both age groups, ChE inhibition $NOEL_{brain}$ = 0.5 mg/kg and $NOEL_{RBC}$ = 0.1 mg/kg/day CPF with repeated exposure. CPFO studies: ChE activity was measured after single or repeated CPFO doses (acute: 0, 0.005 (pups only), 0.01, 0.05, 0.1, 0.5, or 1 (adults only) mg/kg; repeated: 0, 0.01 or 0.5 mg/kg/day). Brain ChE was not inhibited in pups or adults at any CPFO dose in either exposure scenario. In both age groups, ChE inhibition $NOEL_{RBC}$ = 0.1 mg/kg and 0.01 mg/kg/day CPFO with acute and repeated exposures, respectively. Across studies, plasma ChE inhibition was similar to that seen in RBCs. These data indicate that young pups are not more sensitive to ChE inhibition by CPF or CPFO over the lower portion of the dose-response curves.

PS 1325 REPEATED DEVELOPMENTAL CHLORPYRIFOS EXPOSURE INCREASES ENDOCANNABINOID LEVELS IN THE BRAIN OF JUVENILE RATS.

R. L. Carr, A. B. Ward and M. K. Ross. *Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS.*

The endogenous cannabinoids 2-arachidonylglycerol (2-AG) and anandamide (AEA) play vital roles during nervous system development including regulating axonal guidance and synaptogenesis. The degradation of 2-AG and AEA is mediated by monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively. We have previously reported that developmental repeated exposure to 5 mg/kg chlorpyrifos (CPS) results in inhibition of these enzymes in rat forebrain with FAAH activity being more sensitive than cholinesterase (ChE) activity. This greater sensitivity of FAAH than ChE is also present following exposure to lower dosages of CPS (18% ChE as compared to 40% FAAH inhibition at 4 hrs following 1 mg/kg CPS). However, it is not clear if the inhibition of FAAH results in any changes in the level of the endocannabinoids especially at low dosages. To determine this, 10 day old rat pups were exposed daily for 7 days to either corn oil or increasing dosages of CPS (1, 2.5, or 5 mg/kg) by oral gavage. At 12 hrs post exposure to the three dosages, forebrain 2-AG levels were significantly increase by 27%, 44%, and 96% while forebrain AEA levels were significantly increased by 86%, 148%, and 253%. This significant accumulation of 2-AG and AEA could result in activation of endocannabinoid transmission during brain maturation. This could have implications for the appropriate development of the endocannabinoid system leading to permanent alterations in neuronal brain circuits and behavioral responses.

PS 1326 PON1 STATUS MODULATES CEREBELLAR GENE EXPRESSION CHANGES ASSOCIATED WITH DEVELOPMENTAL EXPOSURE TO CHLORPYRIFOS OXON.

T. B. Cole[1, 2], R. P. Beyer[2], T. K. Bammler[2], S. S. Park[1], F. M. Farin[2], L. G. Costa[2] and C. E. Furlong[1]. [1]*Genome Sciences and Medicine, Division of Medical Genetics, University of Washington, Seattle, WA and* [2]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA.*

Chlorpyrifos (CPF) affects brain development and function via multiple mechanisms. Individuals carrying the Q192 allele of paraoxonase (PON1) are more sensitive than those carrying the R192 allele to the effects of CPF and chlorpyrifos oxon

(CPO) exposure, especially if combined with lower plasma levels of PON1. We examined the neurodevelopmental effects associated with low-level chronic exposure of mice to CPO during postnatal development. PON1 knockout (PON1-/-), wild-type (WT) and humanized tgHuPON1Q192 and tgHuPON1R192 transgenic mice were used to determine whether PON1 status modulated the effects of CPO exposure on gene expression in the cerebellum. Neonatal mice were exposed to CPO (0, 0.15, 0.18, 0.25, 0.35 or 0.50 mg/kg/d) daily from PND4-21. Chronic CPO exposure resulted in dose-related decreases in brain acetylcholinesterase activity by PND22 in PON1-/- and tgHuPON1Q192 mice, but not in WT or tgHuPON1R192 mice. Gene expression was measured on PND22 using Affymetrix Mouse Genome microarrays. All four genotypes exposed to CPO (0.35 or 0.50 mg/kg/d) showed significant differences in gene expression compared with controls. Pathway analysis and Gene Set Analysis revealed multiple pathways and gene sets affected by CPO exposure, including genes involved in mitochondrial dysfunction, oxidative stress, neurotransmission, and nervous system development. Changes in gene expression were modulated differentially by the two Q192R alloforms, with the most overlap in significantly-enriched gene sets occurring between the tgHuPON1Q192 and PON1-/- mice. These findings indicate that neonatal CPO exposure is associated with wide-ranging effects on gene expression in the brain, and that PON1 status can modulate these effects, even when PON1 levels are low during early development. Supported by ES11387, ES09601/EPA-R826886, ES09883, ES04696, ES07033.

PS 1327 CHLORPYRIFOS AND CHLORPYRIFOS OXON ALTER RYANODINE RECEPTOR FUNCTION.

Y. Niknam, A. Ghogha, P. Lein and I. Pessah. *Molecular Biosciences, School of Veterinary Medicine, UC Davis, Davis, CA.*

An accepted mechanism of organophosphorus (OP) insecticide-induced neurotoxicity is inhibition of acetylcholinesterase (AChE), the enzyme that hydrolyzes the neurotransmitter acetylcholine. However, several neurotoxic actions of OPs cannot be directly attributed to AChE inhibition. In a screen of 250 environmental chemicals, chlorpyrifos (CPF), a widely used OP, was identified as a potent modulator of type 1 and type 2 ryanodine receptors (RyR1 and RyR2, respectively), broadly expressed ion channels that regulate the release of Ca2+ from endoplasmic/sarcoplasmic intracellular calcium stores. We further investigated the mechanism and specificity by which CPF, its major bioactive metabolite chlorpyrifos oxon (CPFO), and the predominant breakdown product trichloro-2-pyridinol (TCP) influence RyR1 and RyR2 function using [3H]ryanodine receptor binding analysis, western blotting to detect RyR phosphorylation and electrical recordings of primary sympathetic neurons cultured on multi-electrode arrays (MEAs). Radioligand binding assays performed at 37oC indicated a non-monotonic concentration-effect relationship with low μM concentrations of CPF and CPFO maximally enhancing RyR1 occupancy by 2.4- and 7.5-fold, respectively. CPF and CPFO showed significantly less activation of RyR2 at 37°C (1.3- and 3-fold of control), whereas 1μM CPF was more efficacious toward RyR2 at 25oC (3.3-fold of control). TCP showed negligible activity towards RyR1 and RyR2 under either experimental condition. Western blotting of microsomes treated with <20μM CPF did not show differences in RyR1 phosphorylation at serine-2844 compared to solvent controls. Acute exposure to CPF and CPFO, but not TCP, increased spontaneously activity in cultured sympathetic neurons (2, 3 and 4.5 fold of control at 1, 10 and 100nM CPF; 3 and 3.3 fold of control at 1 and 10nM for CPFO). These data identify RyRs as a sensitive target of CPF and CPFO that may contribute to non-AChE-mediated OP neurotoxicity. Sponsored by NIH (P42 ES04699 to INP and R01 ES16308 to WKA and PJL).

PS 1328 OCCUPATIONAL PESTICIDE EXPOSURE AND NEUROBEHAVIORAL DEFICITS IN AN ADOLESCENT POPULATION.

D. Rohlman[1], A. Ismail[2], J. R. Olson[3], O. Hendy[3] and G. Abdel Rasoul[3]. *[1]CROET, Oregon Health & Science University, Portland, OR, [2]Menoufia University, Shebin Elkom, Egypt and [3]State University of New York at Buffalo, Buffalo, NY.*

Agricultural workers, both adults and adolescents, are at risk for many occupational hazards including workplace injuries and exposure to pesticides. Pesticides are thought to pose a considerably higher risk to children than to adults, yet little is known about the extent or magnitude of health problems related to occupational exposure to pesticides in children and adolescents. Experimental animal studies indicate that the developing brain is more susceptible to the neurotoxic effects of organophosphorus (OP) pesticides than the adult brain, and low-level exposures to OP pesticides cause significant neurobehavioral deficits in animal research. Adolescents in Egypt are hired seasonally to work as pesticide applicators for the cotton crop. The pesticide application to the cotton crop is highly regulated and standardized across Egypt, and has been limited primarily to organophosphorus pesticides, generally chlorpyrifos, in recent years. Adolescent applicators and Controls completed a battery of neurobehavioral tests, an occupational and symptoms questionnaire and a medical exam. Blood and urine samples were collected to evaluate biomarkers of OP exposure. Thirty two percent of Applicators had plasma butyrylcholinesterase (BuChE) activity lower than normative data, compared to 5% of Controls. Applicators also had significantly higher levels of trichloro-2-pyridinol (TCPy), a chlorpyrifos-specific metabolite in their urine ($p < 0.05$) and decreased neurobehavioral performance compared to Controls. Applicators performed worse on 10 out of 12 of the neurobehavioral tests, significant differences ($p < 0.05$) were found on Match-to-Sample, Symbol-Digit, Finger Tapping, and Block Design. Work practice questionnaires indicate limited use of personal protective equipment. This study provides data to characterize exposure and identifies deficits in an adolescent population occupationally exposed to pesticides. (NIH R01 ES016308)

PS 1329 BEHAVIORAL DEFICITS IN EGYPTIAN APPLICATION TEAMS WITH CHRONIC ORGANOPHOSPHORUS PESTICIDE EXPOSURES.

W. K. Anger[1], F. M. Farahat[2], P. J. Lein[3], J. R. Olson[4] and D. S. Rohlman[1]. *[1]CROET, Oregon Health & Science University, Portland, OR, [2]Menoufia University, Shebin Elkom, Menoufia, Egypt, [3]University of California, Davis, CA and [4]State University of New York at Buffalo, Buffalo, NY.*

Chronic exposure to organophosphorus pesticides (OPs) has been consistently associated with deficits on neurobehavioral tests in workers using pesticides as compared to controls in most of the 24 published studies of these working populations. While years of applying OPs have correlated with effect in 3 studies, a dose-response relationship has not been identified, leading some to doubt the association. We identified a population of pesticide applicators in Egypt primarily exposed to one OP, chlorpyrifos, with limited exposure to other pesticides. Application teams are composed of engineers (who do not typically enter the fields during applications), technicians (who walk side-by-side with the applicators in the fields), and applicators (highest exposures) who are typically seasonal workers. A chlorpyrifos-specific metabolite measured in urine confirmed the pattern of lower to higher exposures across these job categories in this population. Trailmaking, a timed test which requires a broad range of cognitive capabilities, was administered to 60 technicians, 64 engineers, and 20 controls who did not work in agriculture (very low exposures) 3 times during the OP application season and again 1.5 months after applications had ended. Time to complete Trailmaking B improved in each group with a mean overall improvement of 42 secs. A consistent dose-response relationship was seen in test performance speed across all 3 sessions: Controls had the best performance, engineers had slower performance (mean 28 sec slower than controls), and technicians (highest-exposed) had the slowest performance (mean 44 sec slower than controls). When performance was again tested at 1.5 months after applications ended, technicians were a mean of 21 sec slower than engineers, suggesting that the differences between the groups were persistent. Supported by NIH R01 ES016308 to WKA and PJL.

PS 1330 BIOMARKERS OF NEUROTOXICITY IN A RAT MODEL OF OCCUPATIONAL CHLORPYRIFOS (CPF) EXPOSURE.

N. Hussainzada[1, 2], D. Jackson[2], D. Bruun[3], D. Milatovic[4], J. Lewis[2], C. Banks[3], M. Aschner[4], R. Browne[5], J. R. Olson[5] and P. Lein[3]. *[1]Orise Postdoctoral Fellow, Fort Detrick, MD, [2]USACE HR, U.S. Army, Fort Detrick, MD, [3]University of California, Davis, CA, [4]Vanderbilt University, Nashville, TN and [5]State University of New York at Buffalo, Buffalo, NY.*

Identifying individuals at risk for cognitive deficits from repeated low-dose exposure to organophosphorous pesticides (OP) is limited by lack of predictive biomarkers. To address this, we developed a rat model of OP-induced neurotoxicity based on CPF exposure patterns in Egyptian pesticide applicators. Adult male Long Evans rats exposed to CPF (3 or 10 mg/kg/d, s.c.) exhibit significant dose-dependent deficits in Pavlovian fear conditioning at 21 but not 4 or 10 days. Tissues from these animals were used for transcriptomic profiling of the hippocampus in parallel with quantification of peripheral and central biomarkers of CPF exposure, inflammation and oxidative stress. Hippocampal genes differentially regulated by CPF were significantly enriched in biological pathways relevant to synaptic plasticity, cell-to-cell signaling, cell death, inflammation and oxidative stress. CPF also altered

urinary TCPy levels, blood and brain cholinesterase activity, urinary and brain F2-isoprostanes and expression of multiple oxidative stress and inflammatory cytokine biomarkers in brain and blood. Endpoints correlating in a dose- and time-relevant manner to CPF-induced cognitive deficits will be tested as candidate biomarkers in Egyptian pesticide applicators. Opinions, interpretations, conclusions, and recommendations are those of authors and are not necessarily endorsed by the US Army or NIH. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals (NRC 1996) in facilities that are fully accredited by AAALAC. The research described herein was sponsored by the US Army Medical Research and Materiel Command, Military Operational Medicine Research Program and NIH (ES16308).

PS 1331 ORAL EXPOSURE TO THE PESTICIDE CHLORPYRIFOS IN TG2576 MICE, AN ANIMAL MODEL OF ALZHEIMER'S DISEASE.

T. Colomina[1, 2], J. G. Salazar[1], I. Reverte[1, 2], M. Cabré[1], F. Sánchez-Santed[3] and J. L. Domingo[1]. *[1]Toxicology, Rovira i Virgili University, Reus, Spain, [2]Psychobiology CRAM, Rovira i Virgili University, Tarragona, Catalonia, Spain and [3]Neuroscience, University of Almeria, Almeria, Spain.*

Chlorpyrifos (CPF) is a pesticide widely used in intensive agriculture. A number of studies have shown its delayed neurotoxic effects in mammals. In turn, some epidemiological investigations point to a possible relation between CPF exposure and neurodegenerative diseases. In a previous study, we demonstrated an increase of beta-amyloid production long after acute exposure to CPF. The aim of the present study was to assess potential changes in learning and memory of Tg2576 mice carrying the Swedish APP mutation for Alzheimer's disease and their corresponding wild controls, as well to determine the brain levels of beta-amyloid, 2 and 6 months after repeated oral doses of CPF. Eight doses of 25 mg/kg of CPF were given by gavage to Tg2576 mice. General status, body weight, acetyl cholinesterase (AChE) inhibition, behavioral changes, motor coordination in a rotarod, and learning and memory in a modified version of the Morris water maze test were studied. Beta-amyloid (1-40 and 1-42) levels were also measured in brain cortex and hippocampus. A transient decrease in body weight was observed during treatment. Repeated oral exposure to CPF deteriorated motor coordination in wild type mice, while Tg2576 mice performed as badly as the respective controls. Acquisition learning patterns in a water maze was altered in both CPF-exposed Tg2576 mice and wild animal. Moreover, beta-amyloid levels increased in the brains of treated Tg2576 mice two months after CPF exposure. In conclusion, differential genotype behavioral changes emerged after repeated oral exposure to CPF. It raises concern on organophosphate pesticide exposure, as well as neurodegenerative risks for exposed humans.
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PS 1332 EFFECTS OF DIAZINON AND DIAZOXON ON NEURONAL DIFFERENTIATION.

D. Pizzurro[1], G. Giordano[1], K. Dao[1] and L. G. Costa[1, 2]. *[1]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and [2]Department of Human Anatomy, Pharmacology, and Forensic Science, University of Parma Medical School, Parma, Italy.*

Increasing evidence from animal and in vitro studies suggests that organophosphorous insecticides (OPs) may be developmental neurotoxicants. Aside from causing acetylcholinesterase inhibition, many OPs have been shown to affect neuronal differentiation, target glial cells, and elicit oxidative stress. Diazinon (DZ) is one of the most widely used OPs. The effects of DZ and its active metabolite, diazoxon (DZO), on neuronal differentiation are the focus of this project. The overarching hypothesis of this project is that DZ and DZO increase oxidative stress in astrocytes and subsequently impair neuronal development by disrupting glial-neuronal interactions. Astrocytes are known to foster neuronal development, and exposure to certain neurotoxicants has been previously shown to impair this ability. Oxidative stress in primary cortical astrocytes was measured by the oxidation of 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) by reactive oxidative species (ROS) produced by varying concentrations of DZ or DZO (0, 0.1, 1, 5, 10 μM). Sub-cytotoxic concentrations of DZ or DZO caused a 1.5 to 2-fold increase in ROS compared to untreated controls. This effect is comparable to that caused by exposure to hydrogen peroxide. Astrocytes previously exposed to DZ (0.1, 1, 10 μM) for 24 hours were incubated with primary rat hippocampal neurons for an additional 24 hours, and neurite outgrowth was measured. Preliminary results show a 30% decrease in longest neurite length of neurons incubated with astrocytes previously exposed to 10 μM DZ as compared to untreated controls. Hippocampal neurons directly exposed to 10 μM DZ or DZO in astrocyte-conditioned medium exhibit a non-significant decrease in neurite length. Taken together, these results suggest that by eliciting oxidative stress in astrocytes, DZ and DZO can beget neurotoxicity by impairing astrocytes' ability to foster neuronal development. (Supported in part by ES007032 and PO1ES009601)

PS 1333 ORGANOPHOSPHATE (OP) PESTICIDES INDUCE OXIDATIVE STRESS AND OXIDATIVE DNA DAMAGE IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS.

J. Muniz[1], L. A. McCauley[2] and G. E. Kisby[3]. *[1]School of Nursing, University of Pennsylvania, Philadelphia, PA, [2]School of Nursing, Emory University, Atlanta, GA and [3]Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR.*

Epidemiological studies indicate that pesticide exposure is a significant risk factor for neurodegenerative disease (e.g., Parkinson's). Our previous studies indicate that oxidative stress and DNA damage are significantly increased in agricultural workers who used organophosphate (OP) pesticides (Muniz et al., Toxicol Appl Pharmacol 227:97-107, 2008). Oxidative stress and DNA damage may be important mechanisms that link chronic exposure to pesticides with neurodegenerative disease. To determine if OP pesticides induce their effects on human neurons through similar mechanisms, dopaminergic human SH-SY5Y neuroblastoma cells were treated with azinphos-methyl (AZM) (10 μM to 350 μM) for 24-48h and examined for cell viability, oxidative stress and DNA damage [double-strand breaks (DSB), single-strand breaks (SSB) and 8-oxodeoxyguanosine DNA lesions]. Cell viability and glutathione (GSH) levels were significantly reduced ($p<0.0001$) by AZM in a dose-dependent manner and this correlated with increase levels of reactive oxygen species. Pre-treatment with a GSH inhibitor (i.e., BSO) significantly reduced the viability ($p<0.0001$) of AZM treated SH-SY5Y cells whereas pre-treatment with N-acetylcysteine, vitamin E or alpha-phenyl-tert-butylnitrone (PBN) primarily preserved cell viability ($p<0.0001$). These studies demonstrate that AZM induces neuronal cell death through an oxidative stress-mediated mechanism. DNA damage studies (Neutral Comet and Alkaline Comet assay with and without fpg) showed a dose-dependent increase in oxidative DNA damage ($p<0.0001$), but not DSB in AZM treated SH-SY5Y cells. These findings indicate that oxidative stress and oxidative DNA damage are consistent features in both agricultural workers who used OPs and human neural cells treated with an OP pesticide.

PS 1334 GLOBAL GENE EXPRESSION ANALYSIS OF THE CENTRAL NERVOUS SYSTEM OF SARIN EXPOSED RATS (SIX MONTHS POST TREATMENT) SUGGESTS EVOLVING MOLECULAR PATHWAYS.

M. B. Abou-Donia[2] and T. V. Damodaran[1, 2]. *[1]Biology, North Carolina Central University, Durham, NC and [2]Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

We carried out a global gene expression study on the effects of a single intramuscular (i.m.) dose (0.5x LD(50)) of sarin (O-isopropyl methylphosphonofluoridate), also known as nerve agent GB,) in the total brain of male Sprague-Dawley rats at six months post-treatment. Sarin inactivates the enzyme AChE which is responsible for the breakdown of the neurotransmitter acetylcholine (ACh), leading to its accumulation at ACh receptors and overstimulation of the cholinergic system. Control rats were treated with normal saline. Total RNA was extracted and tested for its quality using northern blotting and agilent RNA analysis. The microarray experiment was carried out at NIEHS microarray facility and the data obtained indicated the identification of genes with altered levels of mRNA expression for following categories: A) Genes related to endothelial function, B) genes related to neuronal survival and neurite growth (belonging to SRY box family), C) genes relating to mitochondrial synthesizing and contracting proteins, D) endoplasmic reticulum (ER) membrane proteins, E) endothelium-derived hyperpolarising factor related gene, F) Choline catabolism related gene, G) methyl-CpG binding domain protein related gene, H) major histocompatibility complex (MHC) related genes, I) acetyltransferase complex related gene, J) Interferon related gene,and K) coiled-coil-helix-coiled-coil-helix domain containing protein. Comparison and critical analysis of gene expression data from our several previous studies strongly suggest the evolution of molecular pathways related apoptosis, neuronal survival, and immune response. Other data from histology, neurobehavioral, and biochemical studies on sarin exposure support such an evolving trend in aging mice after sarin exposure.

PS 1335 *IN VITRO* EVALUATION OF INHIBITION OF BUTYRYLCHOLINESTERASE AND NEUROPATHY TARGET ESTERASE CAUSED BY THE ENANTIOMERS OF METHAMIDOPHOS IN BLOOD OF HENS.

G. L. Emerick[1, 2], R. V. Oliveira[2], K. A. Belaz[2], M. Gonçalves[1] and G. H. DeOliveira[1]. [1]*Department of Natural Active Principles and Toxicology, University Estadual Paulista, Araraquara, Sao Paulo, Brazil and* [2]*Department of Chemistry, Universidade Federal de São Carlos, Sao Carlos, Sao Paulo, Brazil.*

Compounds containing an asymmetric center can exist in two isomeric forms known as enantiomers. About 25% of pesticides in current use are chiral, and this percentage is still increasing as more pesticides with complex structures are introduced into application. These enantiomers usually have the same physical and chemical properties, however, often different biological activity. Methamidophos, which has an asymmetric center at the phosphorus atom, is one of organophosphate pesticides in the broader spectrum of action and also the highest toxicity. Thus, the objective of this work was to evaluate the in vitro IC50 of isoforms of methamidophos in relation to plasma cholinesterase (BChE) and neuropathy target esterase in lymphocytes (LNTE) of hens. For determination of BChE and LNTE activity was used the method of Ellman et al. (1961) and Schwab and Richardson (1986), respectively, supplemented with different concentrations of methamidophos in order to estimate the constant of enzyme inhibition and IC50. The form (+)-Methamidophos had lower IC50 for the BChE (2.2 ± 0.40 mM). The racemic form showed intermediate IC50 (4.3 ± 0.23) and Ki (2.4 mM-1). The (-)-Methamidophos had the highest IC50 (6.4 ± 0.30) and lowest Ki (1.6 mM-1), therefore, less toxicity in vitro for the BChE. Regarding LNTE activity, the inhibitory concentrations have shown that the form (+)-Methamidophos had lower IC50. The positive form of methamidophos showed an IC50 value of approximately 6 times smaller than the negative form. These results showed significant differences in toxicity among the three isoforms of methamidophos in relation to BChE and NTE and that it is necessary to undertake further studies in order to reduce the toxic effects on non target species. Animal Committee of Ethics, Resolution: 24/2009. Financial support: FAPESP, grant number 09/51048-8.

PS 1336 CLUSTERING OF SERINE HYDROLASES BY SIMILARITIES OF INHIBITION PATTERNS TOWARD THE 4 STEREOISOMERS OF ISOMALATHION AGREES WITH PHYLOGENETIC PROXIMITIES INFERRED FROM SEQUENCE ALIGNMENTS.

S. J. Wijeyesakere[1], S. Jianmongkol[1], J. A. Doorn[1], B. R. Marable[1], C. M. Thompson[2], I. I. Baskin[3], V. A. Palyulin[3], G. F. Makhaeva[4] and R. J. Richardson[1]. [1]*Toxicology Program, Department of Environmental Health Sciences, University of Michigan, Ann Arbor, MI,* [2]*Department of Pharmaceutical Sciences, University of Montana, Missoula, MT,* [3]*Department of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russian Federation and* [4]*Institute of Physiologically Active Compounds, Russian Academy of Sciences, Chernogolovka, Russian Federation.*

The purpose of the present study was to test the hypothesis that the relative stereoselectivity of serine hydrolases toward inhibition by the 4 stereoisomers of isomalathion is reflected in the phylogenetic order inferred from the aligned sequences of each hydrolase. Bimolecular rate constants of inhibition (ki) of each of the 4 stereoisomers of isomalathion were determined for human (Hu), bovine (Bo), rat (Ra), chicken (Ck), electric eel (Ee), and Torpedo californica (Tc) acetylcholinesterase (AChE); equine (Eq) and Hu butyrylcholinesterase (BChE); and Bo cholesterol esterase (CholE). A scatterplot matrix (Systat 13.00.05) of normalized ki values showed that the order of similarity of serine hydrolases was HuAChE = BoAChE > RaAChE > CkAChE > EeAChE = TcAChE > EqBChE = HuBChE > BoCholE. Moreover, neighbor-joining cluster analysis (Statistica 9.1) of normalized ki values generated a dendrogram with the same ordering as that observed in the scatterplot matrix. Finally, these orderings were in accord with the phylogenetic order inferred from maximum likelihood sequence alignments (ClustalX2 and GeneDoc 2.4). The results suggest that isomalathion stereoisomers may be useful probes of active site topologies and phylogenetic relationships among diverse serine hydrolases. Supported in part by NIH T32 ES07062.

PS 1337 AGE-RELATED BEHAVIORAL EFFECTS OF METHOMYL IN BROWN NORWAY RATS.

R. C. MacPhail, K. A. Jarema and P. M. Phillips. *NHEERL, U.S. EPA, Research Triangle Park, NC.*

Methomyl is a cholinesterase-inhibiting carbamate pesticide that is used in the field on cotton and a variety of fruits and vegetables. Concerns have been raised generally about age-related differences in susceptibility to cholinesterase-inhibiting pesticides, especially for children. This experiment, part of a larger program on life-stage susceptibility, compared methomyl effects on the behavior of adolescent, young-adult and middle-age male Brown Norway rats (Charles River, N=24/age) that were initially 1, 4 or 12 months of age, respectively. Another group of 12-month rats (NIA) was included to evaluate vendor differences. Testing occurred at weekly intervals in photocell devices that recorded horizontal and vertical locomotor activity during 30-min sessions. Methomyl (ChemService) was dissolved in deionized water and given orally in doses of 0 (i.e., vehicle), 0.5, 1.25, 2.0 and 2.75 mg/kg, 15-min before a test session. Order of dosing was counterbalanced so that each rat received all treatments. Following vehicle, horizontal activity was highest in young adults and roughly equal in adolescent and middle-age rats; vertical activity was highest in young adults, slightly lower in adolescents, and considerably lower in middle-age rats. Methomyl produced dose-related decreases in both horizontal and vertical activity. Adolescent rats were least affected. ED50s for horizontal activity were ca. 5.72, 2.43 and 2.19 mg/kg for the adolescent, young-adult and middle-age rats, respectively. Corresponding ED50s for vertical activity were 4.41, 2.20 and 1.85 mg/kg. Middle-age rats from NIA were similarly affected (horizontal ED50=1.96 and vertical ED50=1.61 mg/kg). These results indicate aging increases susceptibility to methomyl in Brown Norway rats; they also emphasize the importance of evaluating pesticide effects at multiple life stages in order to make informed inferences regarding susceptibility and risk. (This is an abstract of a proposed presentation and does not reflect US EPA policy.)

PS 1338 AGE-RELATED DIFFERENCES IN ACQUISTION, STEADY-STATE PERFORMANCE AND CARBARYL EFFECTS ON THE OPERANT BEHAVIOR OF BROWN NORWAY RATS.

K. A. Jarema, P. M. Phillips and R. C. MacPhail. *NHEERL, U.S. EPA, Research Triangle Park, NC.*

The rapid increase in older adults in the population highlights the importance of understanding the role of aging in susceptibility to environmental contaminants. As part of a larger program on life-stage susceptibility, this experiment determined the effect of the carbamate pesticide carbaryl on the operant performance of young-adult, middle-age and senescent male Brown Norway rats. Rats (Charles River) were 4, 12, and 23-24 months old at the start of the experiment, weight-maintained, and trained to perform under a multiple variable-interval (VI) 10-sec variable-interval 100-sec schedule of food reinforcement during 45-min test sessions. Once performances stabilized, one group (n=10-12/age) received each week a dose of carbaryl (3, 6.5 and 10 mg/kg) or corn oil, p.o., 30-minutes before testing. Doses were administered in a mixed order. A second group (n=11-12/age) received weekly carbaryl (10 mg/kg) and corn oil, p.o., at 30, 60, 120 or 240 minutes before testing; treatment times were also arranged in a mixed order. During acquisition, initial response rates for the senescent rats were lower than the young-adult rats, but increased to rates higher than young adults as performance stabilized. Acquisition for middle-age rats resembled young-adult rats early in training, and senescent rats late in training. Carbaryl produced dose-dependent decreases in responding, with the exception of 6.5 mg/kg in the middle-age rats. Senescent rats were overall most affected by carbaryl, especially under VI 100- sec following the lowest dose. Differences in performance due to age after the highest dose (10 mg/kg) were statistically insignificant. Senescent rats, however, took the longest to recover following 10 mg/kg. Age-related differences in carbaryl effects demonstrate the importance of using a range of ages in order to make informed inferences about toxicant susceptibility and risk. (This is an abstract of a proposed presentation and does not reflect US EPA policy.)

PS 1339 CARBARYL NEUROTOXICITY ACROSS THE LIFE-SPAN OF THE BROWN-NORWAY RAT.

V. C. Moser, P. M. Phillips and K. L. McDaniel. *Toxicity Assessment Division, U.S. EPA, Research Triangle Park, NC.*

Demographics show that the proportion of older adults is increasing every year. While there has been considerable attention paid to potential sensitivity of the young to environmental chemicals, there is much less known about the relative vulnerability of the aged. Differences in response could be due to altered sensitivity as a result of toxicokinetic and/or toxicodynamic changes. We systematically compared the dose-response data across the age continuum for neurotoxicity produced by carbaryl, an *N*-methyl carbamate that produces acute toxicity via inhibition of acetylcholinesterase (ChE). Male Brown-Norway rats were tested on postnatal day 18 (PND18), and at 1, 4, 12, and 24 months of age (n=9-10/dose/age), and were orally gavaged with vehicle (corn oil), 3, 7.5, 15, or 22.5 mg/kg carbaryl. Horizontal (all ages) and vertical (rearing; adults only) motor activity was measured for 20 min beginning 15 min after dosing. Within 5 min after the activity session, rats were sacrificed and brain and RBC were collected for subsequent assay of ChE

activity. The dose-response curves for brain ChE and horizontal activity were significantly different across ages. For brain ChE, the PND18 pups were more affected than the other ages. On the other hand, RBC ChE was similarly decreased across all ages. Older rats (12 and 24 mo) were more sensitive to decreases in horizontal activity, especially at the higher doses, whereas the 4 mo old rats were least sensitive. The same pattern was seen with vertical activity. In both control and treated rats, variability of the behavioral measures was greatest in the youngest and oldest rats, unlike ChE measures which showed similar variability across ages. Thus, the youngest rats were the most sensitive to the ChE-inhibiting effects of carbaryl, but older rats showed the most motor activity depression. The explanation for the dissociation between ChE inhibition and motor activity changes across ages is unclear.
This is an abstract of a proposed presentation and does not reflect US EPA policy.

PS 1340 EFFECTS OF GESTATIONAL & LACTATIONAL EXPOSURE TO THE PYRETHROID PESTICIDE DELTAMETHRIN ON NEURAL DEVELOPMENT.

M. Pine, B. Reimers, R. Mariano, D. DeVillier, T. Wagner and G. Ko. *Texas A&M University, College Station, TX.*

Due to concerns over the toxicity of organophosphate and organochlorine pesticides, their use has decreased while that of less toxic alternatives such as pyrethroid pesticides has steadily grown. However, there is little data as to pyrethroid effects on neural development after low dose exposure during pregnancy and lactation. The present study measured the impact of the type II pyrethroid, deltamethrin (DM), on brain development using the Sprague-Dawley rat model. Females were dosed orally with 5.0 mg/kg/day DM or corn oil beginning on day 1 of pregnancy and continuing throughout lactation (PND21). Another group was exposed to the neurodevelopmental toxin valproic acid (VA; 400 mg/kg) by intraperitoneal injection on embryonic day 12.5 only. DM treatment caused a significant reduction in pup body weight while exposure to VA had no effect. We weighed the cerebral cortex, diencephalon, hippocampus, cerebellar hemisphere, and vermis on PND21. DM treatment resulted in decreased absolute weight of the cerebral cortex and cerebellar hemisphere, but did not affect the relative weight (% of body weight) of these brain regions. Interestingly, the relative weight of the cerebellar vermis from DM exposed pups was greater than that of the controls. VA exposure resulted in a decrease in the absolute weight of the cerebral cortex only. To assess any long term effects on behavior and motor coordination, a minimum of two pups from each litter underwent behavioral (somatosensory and open field) and motor coordination (rotarod) tests on PND90. DM treated animals were less sensitive to sensory stimuli based on their decreased removal of progressively smaller sizes of adhesives. Both VA and DM exposed females spent more time in the center of the open field chamber as compared to controls while males spent less time in the center. No differences were noted in coordination (rotarod and gait analysis). These results suggest that DM exposure adversely affects brain development at doses two times below the current lowest observable adverse effect level of 10.0 mg/kg/day.

PS 1341 AGE-DEPENDENT MODULATION OF SODIUM CHANNEL LEVELS AND CALPAIN ACTIVATION FOLLOWING ACUTE DELTAMETHRIN EXPOSURE.

J. P. Magby[1, 2] and J. R. Richardson[1, 2]. [1]*Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ and* [2]*Graduate School of Biomedical Sciences, RWJMS, Piscataway, NJ.*

Sodium channels (Na(v)) are a critical component of neuronal signaling and a target of pyrethroid pesticides, which function as Na(v) agonists. Previously published work found that the sodium channel agonist alpha-scorpion toxin causes internalization and degradation Na(v) proteins in vitro. These effects were age-dependent, as alpha-scorpion toxin promoted internalization of Na(v) in slices from animals at postnatal day (PND) 12, but not at PND30. However, it is not known if this down-regulation of Na(v) protein occurs in vivo. To determine the relevance of these findings in intact animals, PND 12 and 30 rats (n=3-5) were dosed with deltamethrin (0, 3, or 6 mg/kg) and sacrificed 30 min or 2 hr later for determination of membrane levels of Na(v) by western blot. In PND 12 rats, administration of 3 mg/kg deltamethrin resulted in a 20% reduction of membrane Na(v) levels at 30 min and a 25% reduction at 120 min in the striatum. In contrast, administration of 3 mg/kg or 6 mg/kg deltamethrin to PND30 rats resulted in no reduction in membrane Na(v) levels. However, recent in vitro data has found that the calcium-dependent protease calpain can mediate cleavage of Na(v) protein, rendering membrane levels unchanged while generating a non-functional protein. To determine whether calpain is activated by deltamethrin, we measured spectrin cleavage, an indicator of calpain activation. In PND12 animals deltamethrin exposure did not result in increased spectrin cleavage. Conversely, administration of 3 or 6 mg/kg deltamethrin to PND30 rats resulted in an increase of spectrin cleavage at 30 min by 63% and 212%, respectively. These data suggest that acute deltamethrin exposure results alterations of Na(v) protein by different mechanisms that are age-dependent, but that either mechanism may lead to interruption of neuronal signaling and neurotoxicity. Supported by R01ES015991, P30ES 005022, and a Bristol-Myers Squibb Predoctoral Fellowship.

PS 1342 DELTAMETHRIN INHIBITS HUMAN VOLTAGE-SENSITIVE CALCIUM CHANNEL ISOFORMS EXPRESSED IN XENOPUS OOCYTES.

D. Galluzzo, E. M. Mutanguha, Z. Valentine and S. B. Symington. *Biology and Biomedical Science, Salve Regina University, Newport, RI.*

Low voltage-activated (T-type) calcium channels are involved with a variety of physiological processes including pacemaking activity and repetitive firing in neurons. Human Cav3.1, Cav3.2 and Cav3.3 are three T-type calcium channel isoforms that are expressed in different tissues, possess unique molecular pharmacology, and have different voltage-dependent kinetics. Deltamethrin is a potent CS-syndrome pyrethroid that is frequently used in agricultural and vector control programs to control pests. In this research the effect of deltamethrin was evaluated on Cav3.1, Cav3.2, and Cav3.3 expressed in Xenopus oocytes. The Cav3 isoforms were verified using a combination of restriction enzymes and linearized DNA was used as a template to transcribe cRNA using the mMessage mMachine in vitro transcription kit. Cav3 channel expression was confirmed by two electrode voltage clamp electrophysiology. Concentration-dependent response curves were generated on the relative peak current remaining following perfusion of increasing concentrations of deltamethrin. Deltamethrin was found to inhibit the peak current of all Cav3 isoforms. Concentration-dependent response curves were generated for Cav3.2 and Cav3.3. Preliminary results indicate that deltamethrin is a potent inhibitor of Cav3.2 and Cav3.3. Furthermore, high concentrations of deltamethrin inhibit Cav3.1, however, a complete concentration-dependent response has yet to be established due to inconsistent expression of this clone. Current studies are underway to assess the effects of deltamethrin on the voltage-dependent and steady kinetics of Cav3.1 and Cav3.3 in comparison to already compiled data on Cav3.2. Nevertheless, our results indicate a structural specific interaction with deltamethrin on all three Cav3 isoforms.

PS 1343 DIFFERENTIAL SENSITIVITY OF RAT VOLTAGE-GATED SODIUM CHANNEL ISOFORMS TO THE PYRETHROID INSECTICIDE TEFLUTHRIN.

D. M. Soderlund[1], J. Choi[2] and J. Tan[3]. [1]*Entomology, Cornell University, Geneva, NY,* [2]*Neurology, Yale University School of Medicine, New Haven, CT and* [3]*Monsanto Company, St. Louis, MO.*

Voltage-gated sodium channels are important sites for the neurotoxic actions of pyrethroid insecticides in mammals. The pore-forming α subunits of mammalian sodium channels are encoded by a family of 9 genes, designated Nav1.1 - Nav1.9. We used the potent pyrethroid tefluthrin as a probe of pyrethroid sensitivity in assays of rat Nav1.2, Nav1.3, Nav1.6, Nav1.7 and Nav1.8 sodium channels expressed in Xenopus laevis oocytes. All five isoforms were modified by tefluthrin in the resting state. Channel activation by repeated depolarization significantly enhanced the extent of tefluthrin modification of all isoforms except Nav1.8, indicating that tefluthrin binds preferentially to these four isoforms either in the open state or to in other activation-dependent channel states. For Nav1.2 and Nav1.6 channels, the increase in apparent affinity for tefluthrin upon repetitive depolarization was more than 10-fold. The five sodium channel isoforms segregated into two groups on the basis of tefluthrin sensitivity, with Nav1.3, Nav1.6 and Nav1.8 forming the more sensitive group and Nav1.2 and Nav1.7 the less sensitive group. Direct comparison of the sensitivity of Nav1.2 and Nav1.6 channels to resting and use-dependent modification showed that the Nav1.6 isoform was ~15-fold more sensitive to tefluthrin modification. Less extensive data sets for the remaining 3 isoforms show that Nav1.7 channels were somewhat less sensitive than Nav1.2 channels whereas Nav1.3 channels were more sensitive than Nav1.6 channels. Alignment of the amino acid sequences of these five isoforms identified two sequence polymorphisms, located in the S6 transmembrane segments of homology domains I and II, as possible determinants of differential sensitivity of rat sodium channel isoforms to tefluthrin and other pyrethroids.
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PS 1344 CHARACTERIZATION OF DIFFERENTIAL EFFECTS OF ALLETHRIN ON N- AND L-TYPE NEURONAL VOLTAGE-GATED CALCIUM CHANNELS IN DIFFERENTIATED PC12 CELLS.

A. P. Neal[1], S. M. Fox[1], D. Wiwatratana[2], Y. Yuan[1] and W. D. Atchison[1]. [1]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI and* [2]*Neuroscience Program, Michigan State University, East Lansing, MI.*

Pyrethroid insecticides are widely used world-wide in agriculture, malaria control, and as topical insect repellents. Several studies have identified effects on neuronal voltage-gated calcium (Ca^{2+}) channels (VGCCs). Whole-cell voltage-clamp electrophysiology in differentiated PC12 cells was used to determine the IC_{50}/EC_{50} of effects by a well-characterized type I pyrethroid, allethrin (AL) on N- and L-type VGCC subtypes (Cav2.2 and Cav1.2, respectively). Pharmacological agents were used to isolate specific currents. AL exhibited a concentration-dependent inhibition of peak and end N-type VGCC current with IC_{50}'s ~ 10 μM. In contrast, it caused U-shaped concentration-dependent effects on L-type VGCCs, resulting in L-type current peak and end stimulation with EC_{50}'s in the mid-pM range. At higher concentrations (>50 μM), AL did not stimulate L-type current. Additionally, AL altered the V_{50} of activation, but not inactivation, of L-type VGCCs in a concentration-dependent manner, and altered the kinetics of activation and inactivation of both channel subtypes. AL also significantly increased the Ca^{2+} influx associated with L-type VGCC activation, determined by measurements using the ratiometric dye Fura-2. Finally, we observed that modulation of N- and L-type VGCC by AL can have effects on VGCC expression; a 24 hr incubation with 1 μM AL resulted in increased N-type and decreased L-type VGCC expression visualized by immunocytochemistry. Together, these data demonstrate that AL can differentially modulate VGCC function, resulting in altered Ca^{2+} influx, and may have long term effects on VGCC expression. [Supported by NIEHS R01-ES03299]

PS 1345 PYRETHROID EFFECTS ON MUSCARINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN N1E-115 AND PC12 CELLS.

J. M. Diaz Otero[1], A. P. Neal[2] and W. D. Atchison[2]. [1]*University of Puerto Rico at Cayey, Cayey, Puerto Rico and* [2]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Pyrethroid insecticides (PYRs) are widely used and are classified as type I and type II agents. Both primarily target the voltage-gated Na^+ channel, but some studies identified alternative targets of PYRs. Changes in the expression of muscarinic acetylcholine receptors (mAChRs) are observed during developmental PYR exposure. These studies were contradictory; some observed an increase and others a decrease in mAChR expression. We previously observed that PYRs differentially modulate voltage-gated Ca^{2+} channel subtypes, so hypothesized that these discrepancies may be due in part to differential actions on mAChR subtypes. We examined M1 and M2 mAChR expression using fluorescent immunocytochemistry in differentiated (DIF) and undifferentiated (UNDIF) rat pheochromocytoma (PC12) and mouse neuroblastoma (N1E-115) cells. Both models were exposed to the type I and type II PYRs allethrin (AL) and deltamethrin (DM), respectively. Fluorescent staining indicated the expression of surface and cytosolic mAChRs. Both PC12 and N1E cells exhibited differentiation-dependent and -independent effects. In PC12 cells, AL or DM decreased surface M2 expression in UNDIF cells but increased it in NGF-DIF cells. Only DM affected M1 expression in PC12 cells, regardless of differentiation. Conversely, N1E M2 expression was affected in a differentiation-independent manner; both UNDIF and DIF N1E cells had increased M2 expression after DM exposure. However, the effects of AL on M1 expression in N1E cells were dependent on differentiation. In UNDIF N1E cells, M1 expression was unaffected by PYR exposure. In DIF N1E cells, M1 expression significantly increased after exposure to AL, but not DM. Thus, M1 expression in DIF cells of both lines was altered by opposite PYR classes. This suggests that effects of PYRs on mAChR expression are influenced by developmental stage and that these insecticides can exhibit class-specific effects on mAChR subtypes. [Supported by 2R01ES03299 and 1R25NRS06577]

PS 1346 TIME, DOSE, AND STRUCTURE DEPENDENT ACTIONS OF PYRETHROID INSECTICIDES ON RAT THERMOREGULATION.

A. M. Pato[1], C. Sosa-Holt[1] and M. J. Wolansky[1, 2, 3]. [1]*Biological Chemistry (Toxicology), University of Buenos Aires School of Science, Buenos Aires, Argentina,* [2]*Argentine NRC, Buenos Aires, Argentina and* [3]*ARG National Agency for Scientific and Technological Promotion, Buenos Aires, Argentina.*

Pyrethroid insecticides produce changes in thermoregulatory response (TR) in small rodents. In rat, type I-pyrethroids permethrin (PM) and bifenthrin (BIF) produce hyperthermia, and type II cypermethrin (CPM) and deltamethrin (DLM) produce hypothermia. These structure-specific TR effects have been mostly demonstrated using middle-highly effective doses and rectal probe measurements after single oral dosing. In addition, CPM and DLM have been reported to produce biphasic dose-effect relationships: mild hyperthermic effects at low doses, and the expected hypothermia at greater dosages. At present, there is insufficient data to establish if the joint neurotoxicity of pyrethroids is influenced by the structure-specificity of the endpoint selected for study. In order to explore structure- and dose-related actions, we obtained time- and dose-effect relationships for above pyrethroids using mini-transponder technology (BMDS, USA). These telemetry-based chips collect and transmit real-time, subcutaneous body temperature (T) in a stressless fashion. Adult Wistar rats were implanted ~96 h before testing and TR was monitored to determine individual physiological baselines. The testing day, scans were taken at 30 min intervals for 5 hr after a single oral dose (~1-20% LD50; 1 ml/kg) of each compound dissolved in corn oil (N = 6-8 per dose group). The expected type I- and II-like TR patterns were observed at highly effective doses (in mg/kg: PM 150, BIF 12, DLM 9, α-CPM 15). However, a mild increase in T was observed for all pyrethroids at much lower doses regardless of structure. These preliminary results suggest that the dual, type I/II classification only may come into play above a certain threshold dose for a compound-specific toxicogenomic pathway. We plan to test the joint action of low-effective doses of all of these pyrethroids using TR as an endpoint to explore the impact of above findings on cumulative risk estimation.

PS 1347 ROLE OF DOPAMINE TRANSPORTER IN MANEB AND MANCOZEB TOXICITY.

S. Cheng, J. Quinones and C. Cuellar. *Sciences, John Jay College, New York, NY.*

Studies showed that pesticide exposures, such as dithiocarbamates, can potentiate the effects of the neurotoxin MPTP in dopaminergic neurons of mice eventually leading to neurodegeneration. Dopamine transporter (DAT) is a protein known to play a role in MPTP's toxicity by transporting MPP+, the metabolite of MPTP, into dopaminergic neurons. Alpha-synuclein, a DAT-interacting protein, has been known to aggregate into fibrillar cytoplasmic inclusions (Lewy bodies) which has been shown to be a key pathological marker associated with Parkinson's disease. One of alpha-synuclein functions is to mediate the recruitment or maintenance of DAT on the cell surface. Our preliminary data demonstrated that maneb (MB) and mancozeb (MZ), the Mn-containing dithiocarbamates, enhance MPP+-induced cell death in PC12 cells. However, the neurotoxic mechanisms involved in this action are still not clear. Our hypothesis is that MB and MZ increase the interaction of DAT and alpha-synuclein followed by increasing cell surface DAT expression which in turn enhances MPP+ cytotoxicity. PC12 cells were treated with MB (20 uM) and MZ (20 uM) for 1 h at 37°C/5% CO2. After treatments, the biotin-labelled cell surface proteins were isolated and subjected to Western blot analysis for evaluating changes in cell surface DAT expression. The data showed biotinylated DAT was increased (about 200%) after pesticide treatments. Total cell lysates were subjected for co-immunoprecipitation by anti-DAT antibody. Co-immunoprecipitated proteins were subjected to Western blot analysis for alpha-synuclein and DAT. The results showed the interactions of alpha-synuclein and DAT after pesticide treatments were increased about 200%. These results demonstrated that the expression of cell surface DAT and the interaction between DAT and alpha-synuclein are regulated by MB and MZ. Further experiments will be needed to elucidate the direct involvement of alpha-synuclein and dopamine transporter in the potentiated effect of dithiocarbamates in MPP+ induced neuronal cell death.

PS 1348 USE OF NON-BIASED STEREOLOGY TO ESTIMATE THE NUMBER OF TH+ NEURONS IN THE *SUBSTANTIA NIGRA* OF 8 AND 16 MONTH OLD MALE AND FEMALE C57BL/6 MICE REPEATEDLY EXPOSED TO PARAQUAT AND MANEB.

J. P. O'Callaghan[1], K. A. Kelly[1], D. B. Miller[1], R. C. Switzer[2], E. C. Lau[3], A. A. Li[3] and L. J. McIntosh[3]. [1]*Neurotoxicology Laboratories, Centers for Disease Control, Morgantown, WV,* [2]*NeuroScience Associates, Knoxville, TN and* [3]*Exponent, Inc., Menlo Park, CA.*

A proposed animal model for Parkinson's Disease is combined intraperitoneal exposure to paraquat (PQ) and maneb (MB) in C57Bl/6 mice. Stereologic methods were used to estimate tyrosine-hydroxylase positive (TH+) neuron number in the substantia nigra pars compacta (SNpc) of mice exposed to 3 dose levels of PQ+MB postnatally (PND) prior to weaning or both PND and as adults. Four groups of pups were dosed on PND 5-19 with 0/0, 0.3/1.0, 0.06/0.18, or 0.0007/0.0021 mg/kg PQ and MB (PQ/MB), respectively. These pups were divided into 3 sets of

mice, each with 4 dose groups. One set dosed only PND was sacrificed at 8 months of age. Two sets were dosed again as adults twice weekly during weeks 27 to 31 of age with 0/0, 10/30 or 5/15, 0.6/1.8, or 0.0007/0.0021 mg/kg PQ/MB. (The scheduled 10/30 dose was reduced to 5/15 after adult male mortality occurred in one group). One set of PQ+MB groups dosed PND and as adults was sacrificed at 8 months of age, the other at 16 months of age. Brains of all animals were serially sectioned at 50 uM thickness, immunostained for TH, and counterstained for Nissl using thionine. Every 6th section in the SNpc was counted using stereological techniques with Stereo Investigator (MicrobrightField, Inc. Williston, VT) v9.0 software to give a non-biased number of TH+ cells in the SNpc using the optical fractionator method. A treatment-related decrease in SNpc TH+ neuron count was measured in high dose males dosed as pups and as adults and sacrificed at 16 months of age (n=5-6; p=0.06 Tukey-Kramer). There were no differences from controls in TH+ neuron number in females sacrificed at 16 months. At completion of the study, the total number of animals per group will be 8-11.

PS 1349 PARAQUAT AND MANEB ALTER CELLULAR REDOX STATUS BY NON-REDUNDANT MECHANISMS IN SH-SY5Y NEUROBLASTOMA CELLS.

J. Roede and D. P. Jones. *Medicine, Emory University, Atlanta, GA.*

Parkinson's disease (PD) is the most common neurodegenerative movement disorder; however, less than 10% of all cases can be attributed to a genetic component. Therefore, the vast majority of PD cases are considered idiopathic and epidemiological studies have implicated chronic pesticide exposure as contributing to the etiology of PD. Recent epidemiological and in vivo evidence indicates that exposure to the pesticides paraquat (PQ) and maneb (MB) results in an increased risk of developing PD and dopaminergic cell loss, respectively. The purpose of this study was to investigate the mechanism of PQ and MB toxicity and evaluate how these agents alter cellular redox status and cause cell death in vitro. Using SH-SY5Y neurblastoma cells we found that MB was 25 times more toxic than PQ after 24 hours. PQ treatment caused a significant increase in intracellular ROS production; however, MB did not increase ROS production. Also, PQ treatment resulted in significant alterations in the redox state of the thioredoxin/peroxiredoxin system, while MB primarily altered cellular GSH redox. Taken together, PQ and MB alter cellular homeostasis is a non-redundant manner, which potentially exacerbates toxicity when cells are exposed to PQ and MB in combination.

PS 1350 MANEB (*MANGANOUS ETHYLENEBIS[DITHIOCARBAMATE]*) EXPOSURE IN RAT HIPPOCAMPAL ASTROCYTES LEADS TO ACTIVATION OF INTRINSIC APOPTOTIC PATHWAY.

M. Akhtar and L. D. Trombetta. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Maneb is a manganese-containing ethylenebis[dithiocarbamate] widely used fungicide. In general, dithiocarbamates have been shown to produce reactive oxygen species (ROS) and to alter cellular antioxidant homeostasis leading to oxidative stress. In addition, the exposure of pesticides may also be related to chronic disorders of the mammalian central nervous system (CNS). This relationship between dithiocarbamates and neurodegenerative disorders is increasingly being investigated. Previously, we have shown that, rat hippocampal astrocytes exposed to LC50 (13.5μM) concentration of maneb for 24 hours significantly increased lipid peroxidation. Moreover, significant alteration of cellular antioxidants level was also observed. The mode and mechanism of cell death in cultured rat hippocampal astrocytes due to maneb exposure is not yet elucidated. The purpose of this investigation is to elucidate the mechanism of cell death in rat hippocampal astrocytes treated with maneb. Rat hippocampal astrocytes were maintained in Dulbecco's modified Eagle's medium at 37°C, 8.0% CO_2 and supplemented with 10% FBS. Cells at 60-70% confluency were treated with 13.5 μM maneb. Caspase 3 & 7 activities were measured at 4, 8, 12, 16 and 24 hours of exposure. Caspase 9 activity was measured at 12, 16 and 24 hours of exposure. Cells were also harvested after 24 hours exposure for the analysis of cellular manganese metal concentration. Cells seeded in chamber slides were treated with Maneb 13.5 μM and after 24 hours of exposure the TUNEL assay was performed. Caspase 3 & 7 assay showed a significant increase ($P<0.05$) in caspase 3 & 7 activites at all time-points tested. In addition, caspase 9 activity was also significantly increased ($P<0.05$) at all time-points. TUNEL assay showed increased TUNEL-positive cells as compared to control. ICP-OES analysis of manganese cellular level showed a significant increase ($P<0.0001$) in manganese concentration in treated cells when compared to control.

PS 1351 THE NEUROTOXIC EFFECTS OF MANEB ON THE HIPPOCAMPUS OF NRF2 (-/-) MICE.

D. M. Kurzatkowksi and L. D. Trombetta. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Maneb is a ethylene(bis)dithiocarbamate fungicide that is used on a wide variety of crops, including, but not limited to fruit, nut, and vegetable crops, ornamental plants, and sod farms. Environmental exposure to this compound has been shown to cause alterations in oxidative defense mechanisms. This study investigated the neurotoxic effects of maneb on the hippocampus of C57BL/Nrf2 (-/-) knockout and C57BL/Nrf2 (+/+) wild type mice. Nrf2 (Nuclear factor-erythroid 2 related factor 2) is the prime transcription factor needed for the induction of a number of phase II detoxification genes through activation of the antioxidant response element (ARE). This knockout animal model is useful in showing the pro-oxidant effects of the metal-containing maneb. Intraperitoneal dosing took place twice a week for 30 days, at dosages 0, 15, 30, and 60 mg of maneb/kg body weight. Significant decreases in body weight were observed in the 60 mg/kg dosed Nrf2 (-/-) group as compared to the controls. Nrf2 (-/-) mice were also found to have an aberration of the hind limbs at the 30 and 60 mg/kg doses of maneb, suggesting hind limb paralysis. These animals dragged their hind limbs after 2 weeks of treatment. This phenomenon was never observed in the WT groups at any dose. Levels of total glutathione were significantly decreased in Nrf2 (-/-) mice as compared to Nrf2 (+/+) mice. These differences were observed at all treatment doses. Alterations in glutathione levels in the hippocampus and the observation of hind limb paralysis seen in Nrf2 (-/-) animals treated with the fungicide maneb, indicate increased susceptibility to neurotoxic injury. The lack of, or alterations to, the ARE complex are an indication of potential neurological insult and may have important environmental implications.

PS 1352 DEVELOPMENTAL EXPOSURE TO AMITRAZ ALTERS THE SEROTONIN SYSTEM.

M. R. Martínez-Larrañaga, J. Del Pino, M. A. Martínez, E. Ramos and A. Anadón. *Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain.*

Amitraz is a formamidine pesticide. The reported biological activity of the formamidines includes MAO inhibition, inhibition of prostaglandin synthesis, local anaesthetic effects and alpha-2 adrenergic activity. The continue presence of amitraz residues may be of particular concern to children and pregnant women. The objective of the present study was to investigate if perinatal amitraz exposure of rats during gestation and lactation alters serotoninergic neurochemistry in their offspring. Pregnant Wistar rats were orally gaved with amitraz (20 mg/kg) or with corn oil (1.0 ml/kg). The dosing period covered the period from day 6 of gestation through day-10 postnatal. Within each group, a total of 24 offsprings of different litters [12 of them control and 12 experimental (six males and six females)] were randomly culled on the lactation day 10. At 60 days of age, male and female offspring of control and amitraz-treated animals were killed and their brains removed. The frontal cortex and striatum were dissected and analyzed for content of serotonin (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) using a HPLC method with electrochemical detection. Developmental amitraz exposure decreased 5-HT levels of male and female offspring at 60 days of age in the frontal cortex (17% $P<0.001$ and 30% $P<0.001$) and striatum (24% $P<0.01$ and 13% $P<0.05$) respect to corn oil controls. Developmental amitraz exposure also decreased 5-HIAA levels of male and female offspring at 60 days of age in the frontal cortex (22% $P<0.001$ and 8% $P<0.01$) and striatum (10% $P<0.001$ and 11% $P<0.05$) respect to corn oil controls. These results suggest there are long-term alterations in the serotonin system after developmental amitraz exposure. This work was supported by projects Ref. BSCHGR58/08(UCM), Ref.No S2009/AGR-1469(CAM) and Consolider-Ingenio 2010 No.CSD2007-063(MEC), Spain.

PS 1353 SODIUM CHANNEL BLOCKER INSECTICIDES (SCBIS) ARE STATE-DEPENDENT INHIBITORS OF MAMMALIAN VOLTAGE-GATED SODIUM CHANNELS.

R. T. von Stein and D. M. Soderlund. *Entomology, Cornell University, Geneva, NY.*

Sodium channel blocker insecticides (SCBIs) are a novel group of structurally diverse insect control agents that includes two compounds (indoxacarb and metaflumizone) registered for commercial use in the United States. SCBIs interact with voltage-gated Na+ channels in both insects and mammals and inhibit nerve function through a novel mode of action that is distinct from pyrethroid insecticides, which act elsewhere on the Na+ channel protein. Voltage-clamp analyses of Na+

currents have shown that SCBIs have no apparent effect on Na+ channels at hyperpolarized membrane potentials at which channels occupy resting conformations. However, prolonged depolarizations to potentials that promote non-conducting slow-inactivated states increase the affinity for SCBIs, resulting in a slow reduction ("block") of peak Na+ current amplitude that typically develops over a period of several minutes. These results imply that SCBIs act by selectively stabilizing Na+ channels in slow-inactivated conformations. The state-dependent action of SCBIs is functionally analogous to that of local anesthetic, class I anticonvulsant and class I antiarrhythmic drugs, which preferentially block open and fast-inactivated Na+ channels. These drugs bind to the local anesthetic (LA) receptor, which is formed by residues in the S6 transmembrane segments of at least three of the four Na+ channel homology domains that form the inner ion pore. Several lines of evidence suggest that SCBIs and therapeutic Na+ channel blockers may share a common site of action. In particular, mutagenesis experiments show that residues in Na+ channel DIV-S6 identified previously as elements of the LA receptor, also are important determinants of SCBI binding and action. However, the larger molecular size of SCBIs compared to therapeutic drugs acting at the LA receptor suggests that SCBI binding determinants exist outside the LA receptor and still have yet to be determined.
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PS 1354 $GABA_A$ RECEPTOR ANTAGONISTS INCREASE FIRING, BURSTING, AND SYNCHRONY OF SPONTANEOUS ACTIVITY IN NEURONAL NETWORKS GROWN ON MICROELECTRODE ARRAYS (MEAS): A STEP TOWARDS CHEMICAL "FINGERPRINTING".

A. M. Johnstone[1], J. Turner[1], C. Mack[1], L. Burgoon[2] and T. J. Shafer[1].
[1]Integrated Systems Toxicology, U.S. EPA, Research Triangle Park, NC and [2]Research Cores Unit, U.S. EPA, Research Triangle Park, NC.

Assessment of effects on spontaneous network activity in neurons grown on MEAs is a proposed method to screen chemicals for potential neurotoxicity. In addition, differential effects on network activity (chemical "fingerprints") could be used to classify chemical modes of action. To test this, we examined effects of 4 $GABA_A$ antagonists (bicuculline (BIC), lindane, picrotoxin (PTX), RDX) on network activity in neocortical neurons and compared them to verapamil (VER; Ca^{2+} channel antagonist), fluoxetine (FLU; $5HT_3$ reuptake inhibitor) and muscimol (MUS; $GABA_A$ agonist). Concentration-response relationships for effects on network spike rates were determined using MEAs, followed by analysis of bursting patterns and firing synchrony using Neuroexplorer and custom written software, respectively. $GABA_A$ receptor antagonists increased network activity (EC_{50} (μM): BIC, 0.41; lindane, 1.9; PTX, 15.2; RDX, 12.3), while the other compounds decreased it (IC_{50} (μM): VER 6.9; FLU, 5.4; MUS, 0.4). $GABA_A$ receptor antagonists, but not other compounds, also altered #bursts/min, burst duration, and %spikes in bursts. $GABA_A$ receptor antagonists increased synchrony of firing (EC_{50} (μM): PTX 0.045; RDX, 8; lindane, 0.15). By contrast, MUS (IC_{50} = 7.7 nM), VER (IC_{50} = 1.0 uM), FLU (IC_{50} =1.5 uM) decreased synchrony. While additional chemical classes need to be evaluated, these data confirm that MEAs are useful to screen chemicals for neuroactivity and indicate that analysis of chemical effects on spike rates, burst parameters and synchrony may be useful to develop chemical fingerprints. This approach will improve the predictive nature of MEA data from uncharacterized chemicals by providing mode of action information. *This abstract does not reflect Agency policy*

PS 1355 EFFECTS OF FIPRONIL ON THE EEG OF LONG-EVANS RATS.

D. F. Lyke, K. L. McDaniel, V. C. Moser and D. W. Herr.
NB/TAD/NHEERL/ORD, U.S. EPA, Research Triangle Park, NC .

We have reported that the non-stimulus driven EEG is differentially altered by deltamethrin or permethrin (Lyke and Herr, *Toxicologist*, 114(S-1):265, 2010). In the current study, we examined the ability to detect changes in EEG activity produced by fipronil, a phenylpyrazole pesticide that inhibits GABA receptors. Adult male Long-Evans rats were dosed with fipronil (po). Range-finding studies (40 or 50 mg/kg, n=5/dose) were conducted using observations of neurological signs, grip strength, and landing foot splay. The data suggested increased handling reactivity, mild tremors and gait changes, circling, decreased hindlimb grip strength, and increased foot splay. Maximal effects were evident about 5 to 8 hours after dosing. Additional rats were implanted with epidural screw electrodes. After about 1 week recovery, non-restrained animals were gavaged with corn oil and tested for 2 days to allow acclimation. On day 3, the rats were dosed with 1 ml/kg vehicle (corn oil), 25 mg/kg fipronil, or 50 mg/kg fipronil and tested 6 h later. EEG was recorded as 30 segments of 2 s durations, transformed using a FFT, and the spectra averaged. Treatment with 25 and 50 mg/kg fipronil decreased the area (17-25% decrease) associated with Gamma activity when recorded between the visual cortex and frontal cortex. The peak amplitude of this Gamma activity was decreased (20% decrease) by 50 mg/kg fipronil. These results differ from the previously reported increases in Gamma activity following treatment with permethrin (11-25% increase in amplitude-area), and lack of changes after dosing with deltamethrin. The data show that fipronil can alter CNS activity as measured by EEG, and the alterations may differ from some other pesticides. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

PS 1356 TREATMENT OF *CAENORHABDITIS ELEGANS* WITH GLYPHOSATE OR MANCOZEB SUGGESTS SELECTIVE NEURONAL DEGENERATION.

R. Negga, M. Machen, J. Salva and V. A. Fitsanakis. *Biology, King College, Bristol, TN.*

Previous studies demonstrate a positive correlation between high levels of pesticide usage and Parkinson's disease (PD), a disease that preferentially targets dopaminergic (DAergic) neurons. In order to better characterize the potential relationship between two common pesticides and specific neurodegeneration, we chronically (24 hours) or acutely (30 min) exposed two different *Caenorhabditis elegans* (*C. elegans*) strains to varying concentrations (LC_{25}, LC_{50} or LC_{75}) of TouchDown (TD: glyphosate) or Manzate (MZ: manganese/zinc ethylene-*bis*-dithiocarbamate). To further model environmental exposure, additional populations of worms were exposed first to TD for 30 min, followed by a 30-min incubation with varying MZ concentrations. Initially, studies were carried out in NW1229 worms (pan-neuronal::green fluorescent protein [GFP] construct). Pixel counts from fluorescence photomicrographs of worms treated with individual pesticides indicated statistically significant decreases ($p < 0.05$)in the number of green pixels in the nerve ring (primarily DAergic), ventral nerve cord (primarily GABAergic or chonlinergic) or both regions. This was in contrast to data from the dual treatment, where a statistically significant decrease ($p < 0.05$) in pixel number was only observed at 20% MZ. In order to further examine the specificity of neurodegeneration following treatment with these agrochemicals, we used EG1285 worms (GABAergic neurons::GFP construct) to verify GABAergic neurodegeneration. Results indicated a statistically significant decrease in number of green pixels associated with GABAergic neurons in both chronic treatment ($p < 0.01$) paradigms, and in the acute MZ treatment only at 30% ($p < 0.001$). Visual inspection of photomicrographs suggested that the decrease in pixel number was due to morphological changes in ventral nerve cord GABAergic neurons. Taken together, our data suggest that exposure to TD or MZ promotes neurodegeneration, including that of GABAergic neurons, in the model organism, *C. elegans*.

PS 1357 EFFECTS OF ATRAZINE AND ITS METABOLITE DIAMINOCHLOROTRIAZINE ON UNDIFFERENTIATED AND DIFFERENTIATING N27 DOPAMINERGIC CELLS.

Z. Lin, C. A. Dodd, I. I. Georgieva and N. M. Filipov. *Physiology and Pharmacology, University of Georgia, Athens, GA.*

Multiple in vitro and in vivo studies have shown that excessive exposure to the herbicide atrazine (ATR) results in dopaminergic neurotoxicity, suggesting that ATR targets the dopaminergic circuitry preferentially. However, effects of ATR or of its major mammalian metabolite diaminochlorotriazine (DACT) on dopaminergic cell differentiation are unknown. Hence, we sought to determine the impact of ATR and DACT on undifferentiated and differentiating dopaminergic cells. Immortalized dopaminergic cells (N27) were exposed for 24 or 48 h to a concentration range of ATR or DACT with and without the presence of a differentiating agent (dbcAMP) in the exposure medium. Exposure to 300 μM ATR or DACT caused time-dependent cytotoxicity and decreased ATP production in undifferentiated N27 cells. On the other hand, 48, but not 24 h, exposure to 300 μM ATR or DACT increased the ADP: ATP ratio in both undifferentiated and differentiated N27 cell, indicating that longer exposure to ATR or DACT in these cells may be apoptotic. Morphological examination indicated that exposure to ATR (≥ 60 μM) during differentiation decreased soma size as early as 24 h. In contrast, DACT disrupted N27 cell differentiation by decreasing thin neurite formation at concentrations as low as 12 μM, but only after 48 h incubation. These data demonstrate that

exposure to both ATR and DACT has adverse impact on dopaminergic cells, including a disruption of the normal differentiation process. Moreover, it appears that ATR and DACT affect the dopaminergic cell differentiation process differently.

PS 1358 EFFECT OF HEXACHLOROBENZENE(HCB)ON THE NEURONAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS.

C. Addae and E. Martinez-Ceballos. *Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.* Sponsor: R. Uppu.

Embryonic stem (ES) cells are pluripotent cells that can differentiate into all three main germ layers: endoderm, mesoderm, and ectoderm. A number of methods have been developed to differentiate ES cells into specific cell types in culture. In preliminary studies, we have found that encapsulation of ES cells in alginate-based hydrogels increases the efficiency of ES cell neuronal differentiation. Now we have made use of this model system to examine the effect that environmental pollutants have on ES differentiation. One of such pollutants, hexachlorobenzene (HCB), is a synthetic industrial chemical that is bioaccumulative, persistent, and a toxic pollutant in the environment. Exposure to this substance is a public health concern because of its association with a wide range of adverse health effects. It is believed that HCB can interfere with brain development and cause mental disorders such as autism. Thus the goal of this research is to investigate the effect of HCB on neuronal differentiation of mouse ES cells. To achieve this goal, ES cells were grown in suspension in ES medium and treated with 10nM HCB for 24, 48, and 72 hours. Fresh medium was added at the end of the treatments and the resulting embryoid bodies (EBs) were harvested on day 8 of culture. Immunofluorescence analysis was performed to examine the effect of HCB on neuronal differentiation. Results showed that HCB has an inhibitory effect on the neuronal differentiation of the mouse ES cells. Because cell death was prominent in HCB-treated ES cells as compared to controls, the dose-dependent effect of HCB on ES neuronal differentiation was also examined. Our studies suggest that low doses of persistent pollutants may affect the differentiating capabilities of ES cells. The relationship of our findings to neurological disorders such as autism is discussed in this work.

PS 1359 INCREASED FREQUENCIES OF MICRONUCLEATED RETICULOCYTES RELATED TO ALDH2 POLYMORPHISMS IN NON-SMOKING AND NON-DRINKING WORKERS EXPOSED TO STYRENE.

Z. Weng[1], D. Guan[2], X. Zhang[4], P. Zhao[3], Y. Zheng[4] and R. Wang[1]. *[1]Japan National Institute of Occupational Safety and Health, Kawasaki, Japan, [2]Beijing Fengtai Health Inspection Institute, Beijing, China, [3]Beijing Guoji Zhongyi Hospital, Beijing, China and [4]National Institute of Occupational Health and Poison Control, China, Beijing, China.* Sponsor: N. Mei.

To determine the effect of occupational exposure to styrene on frequencies of micronucleated reticulocytes (MN-RETs), we recruited 115 reinforced plastic workers and 90 unexposed healthy individuals (both exposure group and control group were non-smoking and non-drinking). In addition, considering genetic polymorphisms can have an effect on styrene-induced toxicity, we evaluated the associations between aldehyde dehydrogenase 2 (ALDH2) polymorphisms and styrene-induced frequencies of MN-RETs. Results showed that frequencies of MN-RETs were significantly increased in styrene-exposed workers than unexposed individuals. In styrene-exposed workers, variant allele ALDH2 *2 was significantly associated with increased frequencies of MN-RETs. However, no effect was observed of ALDH2 polymorphisms on frequencies of MN-RETs in unexposed individuals. Our data indicated that styrene exposure seems to be associated with genotoxicity, and this toxicity may be modulated by ALDH2 polymorphisms.

PS 1360 MODULATION OF DNA REPAIR CAPACITY BY *XPC* HAPLOTYPES.

C. M. Rondelli[1], J. K. Wickliffe[2] and S. Z. Abdel-Rahman[1]. *[1]NCB & ObGyn, University of Texas Medical Branch, Galveston, TX and [2]Environmental Health Sciences, Tulane University, New Orleans, LA.*

Xeroderma pigmentosum complementation group C protein, encoded by the *XPC* gene, plays a key role in DNA nucleotide excision repair (NER). *XPC* is highly polymorphic, with over 90 single nucleotide polymorphisms (SNPs). These SNPs could alter the levels, structure and function of the encoded XPC protein, thus altering DNA repair capacity (DRC). A comprehensive evaluation of all common *XPC* SNPs on DNA damage-response and DRC has not been performed. We constructed a haplotype map encompassing all *XPC* SNPs with a minor allele frequency ≥0.05, and identified 21 haplotypes that segregated into 6 phylogenetic groups (PGHs A-F). We evaluated the relationship between these groups of haplotypes and DNA damage associated with smoking, using chromosome aberrations (CA) as a biomarker. We found a significant interaction between one group, PGH-C, and smoking for baseline CA (P=0.046). Using mutagen-sensitivity as an intermediate biomarker of cancer risk, we observed significant interactions between smoking and two groups, PGH-D (P=0.023) and -F (P=0.007). To provide mechanistic explanations to our findings, we exposed human lymphoblastoid cells with different *XPC* haplotypes to UV radiation, to generate cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4PP). Using ELISA, we evaluated the effect of different *XPC* haplotypes on the repair of CPD and 6-4PP. We hypothesized that if certain *XPC* haplotypes have functional effects, there would be a correlation between these haplotypes and rate of repair of UV-induced genetic damage. Our preliminary results suggest a preference for the repair of 6-4PP over CPD, and show a significant difference in DRC between different *XPC* haplotypes. The data provides initial mechanistic explanations of the effects of *XPC* haplotypes observed earlier in smokers and support the reported associations between *XPC* SNPs and cancer risk.

PS 1361 DRUG METABOLIZING ENZYME POLYMORPHISMS, RESPONSE TO CHEMOTHERAPY AND SURVIVAL IN LUNG CANCER PATIENTS.

A. O. Ada[1], S. C. Kunak[2], F. Hancer[1], S. Bilgen[1], T. G. Ada[1], V. Karacaoglan[1], S. Suzen[1], S. Alpar[3], M. Gulhan[3], B. Kurt[3] and M. Iscan[1]. *[1]Toxicology, Ankara University, Faculty of Pharmacy, Ankara, Turkey, [2]Pharmacology, Giresun University, Medical Faculty, Giresun, Turkey and [3]Pulmonary Diseases Clinic, Ataturk Pulmonary Diseases and Thoracic Surgery Hospital, Ankara, Turkey.*

Lung cancer is an increasing worldwide public health problem particularly in men. In recent years, several polymorphisms in cytochrome P-450s (CYP)s and glutathione S-transferases (GST)s have been reported to be associated with survival rates of patients with non-small cell lung cancer (NSCLC) but the studies in this regard are scarce and the results are contradictory. Moreover, almost no information is available with respect to the relationship between these polymorphisms and response to chemotherapy in NSCLC patients. In this study, CYP1A1, CYP1B1, CYP2E1*5B, GSTM1, GSTO1, GSTP1 exon 5, GSTP1 exon 6 and GSTT1 polymorphisms were determined in 138 NSCLC patients to evaluate their role in survival and response to first-line platinum based chemotherapy. The polymorphisms did not significantly influence the responses to platinum based chemotherapy. Of the studied polymorphisms only GSTP1 exon 6 variant significantly altered (improved) the survival compared to wild type (p=0.036) with median survival of 22.2 months and 16.1 months, respectively. Multivariate analysis also revealed a significant altered (reduced) adjusted hazard ratio of death associated only with the GSTP1 exon 6 variant genotype of 0.45 (95% confidence interval (95% CI), 0.23-0.89, p=0.022). These results show that a) the studied CYP and GST polymorphisms are not functioning as a predictor of response to first-line platinum based chemotherapy, and b) the GSTP1 exon 6 variant genotype is associated with improved survival in the patients with advanced NSCLC. (Supported by the grants from Research Fund of Ankara University Nos: 2006-08-03-002 HPD, 2007-08-03-005 HPD, 2008-08-03-006 HPD and 10A3336002)

PS 1362 CYP AND GST GENE POLYMORPHISMS IN A TURKISH POPULATION.

M. Iscan[1], V. Karacaoglan[1], S. H. Kunak[2], E. Soydas[1], T. G. Ada[1] and A. O. Ada[1]. *[1]Department of Toxicology, Ankara University, Faculty of Pharmacy, Ankara, Turkey and [2]Department of Pharmacology, Giresun University, Faculty of Medicine, Giresun, Turkey.*

It is well known that cytochrome P-450 (CYP) enzymes are able to metabolize many xenobiotics including drugs, polycyclic aromatic hydrocarbons and aromatic amines to reactive metabolites which could be very toxic and/or carcinogenic. The glutathione S-transferases (GST)s are a family of cytosolic enzymes generally involved in the inactivation of drugs and toxic, electrophilic, xenobiotics. Thus, genetic polymorphisms of these enzymes can have great impact on the interindividual susceptibility to toxicities of xenobiotics. In this study, CYP2E1, GSTM1, GSTO1, GSTP1 and GSTT1 gene polymorphisms were determined in 302 healthy individuals. On the basis of an improved polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) methodology, the frequency of CYP2E1*5B (RsaI), GSTO1 (Ala140Asp), GSTP1 codon 105 and GSTP1 codon 114 polymorphisms were determined. The frequencies of c1/c1 (wild type), c1/c2 (heterozygous variant) and c2/c2 (homozygous variant) CYP2E1 genotypes were 94.7 %, 5.3 % and 0 %, respectively. The GSTO1 genotype frequencies were Ala140/Ala140 48.6

%, Ala140/Asp140 40.6 % and Asp140/Asp140 10.8 %. The GSTP1 genotype frequencies were Ile105/Ile105: 56.0 %, Ile105/Val105: 33.4% and Val105/Val105: 10.6%, and Ala114/Ala114: 84.8%, Ala114/Val114: 14.2% and Val114/Val114: 1.0%. The genetic polymorphism analysis for the GSTM1 and GSTT1 genes was determined simultaneously in a single assay using a multiplex PCR approach. As an internal control exon 7 of the CYP1A1 gene was co-amplified. The frequencies of the deleted GSTM1 and GSTT1 genotypes were 54.6% and 22.2%, respectively. These results reveal that the frequencies of CYP2E1*5B (RsaI), GSTM1, GSTO1, GSTP1, and GSTT1 gene polymorphisms in a Turkish population are similar to European Caucasian populations (Supported by the Research Fund of Ankara University, Grants 2006-08-03-002HPD, 2008-08-03-006HPD and 10A3336002).

PS 1363 INFLUENCE OF GENETIC VARIATION IN THE SEROTONIN TRANSPORTER ON SMOKING CESSATION SUCCESS USING ANTI-DEPRESSANT THERAPY.

M. Quaak[1, 2], C. P. van Schayck[2], D. S. Postma[3], E. J. Wagena[2, 4] and F. J. van Schooten[1]. [1]*Health Risk Analysis and Toxicology, Maastricht University, Maastricht, Netherlands,* [2]*General Practice, Maastricht University, Maastricht, Netherlands,* [3]*Pulmonology, University Medical Center Groningen, Groningen, Netherlands and* [4]*Kiadis Pharmacology Amsterdam, Netherlands.* Sponsor: H. van Loveren.

BACKGROUND: Currently, two forms of antidepressant therapy are used for smoking cessation: bupropion and nortriptyline. Based on their mechanism of action, variations in the serotonin transporter gene (SLC6A4) are expected to influence smoking cessation rates. We therefore investigated whether functional variants in SLC6A4 influence smoking cessation rates and treatment response. METHODS: Subjects participated in a randomized, placebo-controlled trial on the efficacy of bupropion and nortriptyline for smoking cessation. The main outcome measures were prolonged abstinence from smoking after 12 weeks (EOT; end-of-treatment) and at 6- and 12-month follow-up. Subjects were genotyped for three functional variants in SLC6A4: 5-HTTLPR, STin2, and rs25531. RESULTS: Carriers of the 5-HTTLPR high-activity L-variant had significantly higher cessation rates with bupropion compared to placebo (6-months: 38% vs. 16%; 12-months: 31% vs. 14%). Similar effects were observed in the nortriptyline group, and for the rs25531 SNP in both the bupropion and nortriptyline group, although non-significant. The STin2 variant seemed to have little effect. Combining the three variants resulted in increased cessation rates compared to placebo with a combination of high-activity variants for both bupropion and nortriptyline (bupropion: 60% vs. 0% at both 6- and 12-months; nortriptyline: 54% and 46% vs. 0% at 6- and 12-months). CONCLUSIONS: Both bupropion and nortriptyline are more effective in the presence of high-activity variants of the serotonin transporter, probably by blocking the increased serotonin transporter activity, hereby increasing serotonin levels. Prospective studies have to assess if this can improve cessation rates when bupropion and nortriptyline treatment is targeted at individuals based on their genotypes.

PS 1364 TOXICOGENOMICS OF NEVIRAPINE-ASSOCIATED CUTANEOUS AND HEPATIC ADVERSE EVENTS AMONG POPULATIONS OF AFRICAN, ASIAN, AND EUROPEAN DESCENT.

J. Yuan[1], S. Guo[1], D. Hall[1], A. Cammett[1], S. Jayadev[1], M. Distel[2], S. Storfer[1], Z. Huang[1], P. Mootsikapun[3], K. Ruxrungtham[4], D. Podzamczer[5] and D. Haas[6]. [1]*Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT,* [2]*Boehringer Ingelheim GmbH, Ingelheim, Germany,* [3]*Khon Kaen University, Khon Kaen, Thailand,* [4]*Chulalongkorn University, Bangkok, Thailand,* [5]*Hospital Universitari de Bellvitge, Barcelona, Spain and* [6]*Vanderbilt University School of Medicine, Nashville, TN.*

Nevirapine is widely prescribed for HIV-1 infection. We characterized relationships between nevirapine-associated cutaneous and hepatic adverse events (AEs) and genetic variants among HIV-infected adults. We retrospectively identified cases and controls. Cases had experienced symptomatic nevirapine-associated severe (grade III/IV) cutaneous and/or hepatic AEs within 8 weeks of initiating nevirapine. Controls did not experience AEs during >18 weeks of nevirapine therapy. Cases and controls were matched 1:2 on baseline CD4 T-cell count, sex, and race. Individuals with ≤150 CD4 T-cells/mm3 at baseline were excluded. We characterized 123 HLA alleles and 2744 single nucleotide polymorphisms in major histocompatibility complex (MHC) and drug metabolism and transport genes. We studied 276 evaluable cases (175 cutaneous AEs, 101 hepatic AEs) and 587 controls. Cutaneous AEs were associated with CYP2B6 516G→T (OR 1.66, all), HLA-Cw*04 (OR 2.51, all), and HLA-B*35 (OR 3.47, Asians; 5.65, Thais). Risk for cutaneous AEs was particularly high among Blacks with CYP2B6 516TT and HLA-Cw*04 (OR 18.90), and Asians with HLA-B*35 and HLA-Cw*04 (OR 18.34). Hepatic AEs were associated with HLA-DRB*01 (OR 3.02, Whites), but not CYP2B6 genotypes. Associations differed by population, at least in part reflecting allele frequencies. The study indicates polymorphisms in drug metabolism and immune response pathways modulated AE risk. These results suggest fundamentally different mechanisms of AEs: cutaneous, most likely MHC class I-mediated and influenced by nevirapine CYP2B6 metabolism; hepatic, most likely MHC class II-mediated and unaffected by such metabolism. These risk variants are insensitive for routine clinical screening.

PS 1365 AN EVALUATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE HUMAN ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR GENE.

R. Budinsky[1], J. D. Urban[2] and J. Rowlands[1]. [1]*The Dow Chemical Company, Midland, MI and* [2]*ToxStrategies, Inc., Austin, TX.*

The effects of dioxin-like chemicals (DLCs) are mediated through the aryl hydrocarbon receptor (AHR); a key step in activation of this pathway involves heterodimerization with aryl hydrocarbon receptor nuclear translocator (ARNT) and subsequent activation of xenobiotic metabolizing enzymes. The objective of this study was to identify single nucleotide polymorphisms (SNPs) in the human *ARNT* gene that could potentially affect the sensitivity of the AHR response pathway. DNA from 101 human samples from six ethnic populations was sequenced using PCR and resulting exonic SNPs were compared with SNPs described previously in the literature or public databases. Results indicated that 69 samples had at least one SNP, and five samples had two SNPs. Five unique SNPs were identified, three of which were non-synonymous (two were novel). Several previously identified SNPs were not identified in the current study, though the most commonly reported SNP (V189V) in other studies was observed at a frequency of 0.49 in this study. The potential functional consequences of the three non-synonymous SNPs identified in the current dataset appear to be limited as the frequency of occurrence was very low (< 0.02), and because two of the SNPs appear in regions of the protein that are not within a defined functional domain. The remaining SNP (V304M) occurred in the PAS domain of a single sample. Studies have suggested that a single point mutation in this domain can negatively impact transcriptional function and potentially lower ARNT levels. With respect to DLC exposures, this would suggest that carriers of this SNP would be less sensitive to effects. However, loss and/or decrease of ARNT function has also been associated with type 2 diabetes and thus additional research is needed to fully understand the functional impact of the V304M variant in the human population. Overall, results indicate a very limited presence of polymorphisms in functional regions of the human *ARNT*.

PS 1366 SINGLE NUCLEOTIDE POLYMORPHISMS IN THE HUMAN ARYL HYDROCARBON RECEPTOR INTERACTING PROTEIN (AIP) GENE FROM SIX ETHNIC POPULATIONS.

D. Staskal-Wikoff[1], R. Budinsky[2] and J. Rowlands[2]. [1]*ToxStrategies, Inc., Austin, TX and* [2]*The Dow Chemical Company, Midland, MI.*

The biological effects of dioxin-like chemicals (DLCs) involve initial binding to and activation of the aryl hydrocarbon receptor (AHR). The human AHR interacting protein (AIP) is a chaperone protein that complexes with AHR, allowing for AHR ligand-binding complex stabilization. It also interacts with AHR and heat shock protein 90 (HSP90) to form a ligand-free AHR complex that prevents ubiquitination and limits protein degradation. Thus the presence of single amino acid polymorphisms at key functional domains in the AIP protein could affect AHR protein integrity and/or signaling. The objective of this study was to screen 103 human DNA samples from six ethnic populations for single nucleotide polymorphisms (SNPs) within the *AIP* gene that could potentially affect the sensitivity of the AHR response pathway. Of the 103 samples evaluated, 42 samples had at least one SNP, 12 samples had two SNPs, while one sample had three SNPs. Eight unique exonic SNPs were identified in the dataset. Three *AIP* SNPs had not been described previously in the literature or public databases, though these all resulted in synonymous substitutions. Two non-synonymous SNPs were identified; both were found in the African American population samples, and one was found in the Mexican group. One of these (A276V) is located in a defined functional region [the

third tetratricopeptide repeats (TPR 3) domain], while the other (Q228R) was present between the TPR 1 and TPR 2 domains. Prior evidence suggests that a single point mutation within AIP TPR region can negatively affect AIP-protein binding, which could potentially disrupt AIP-HSP90 binding and compromise AHR protein integrity and cellular localization. Although this study found limited evidence of *AIP* SNPs of functional consequence, the results suggest that one or two *AIP* SNPs could result in decreased sensitivity to DLC exposures. Further investigation of the impact of these *AIP* SNPs on AHR signaling is warranted.

PS 1367 ASSOCIATION OF GENETIC VARIATIONS IN ANTIOXIDANT ENZYME GENES WITH DIISOCYANATE-INDUCED ASTHMA IN EXPOSED WORKERS.

B. Yucesoy[1], V. J. Johnson[1], K. Fluharty[1], J. Slaven[1], Z. L. Lummus[2], G. E. Kissling[3], D. R. Germolec[3], M. I. Luster[1] and D. I. Bernstein[2]. *[1]NIOSH/CDC, Morgantown, WV, [2]University of Cincinnati, Cincinnati, OH and [3]NIEHS, Research Triangle Park, NC.*

Diisocyanates are the most common cause of occupational asthma induced by low-molecular-weight agents. It has been shown that diisocyanates induce oxidative stress when they react with specific proteins in human airway epithelial cells and this process contributes to the pathogenesis of diisocyanate-induced asthma (DA). In this respect, variability in the antioxidant defenses may play a role in DA susceptibility. A case-control study was conducted to investigate whether genetic variations within antioxidant enzyme genes, glutathione S-transferases (GSTM1-GSTT1, GSTM3, GSTP1), manganese superoxide dismutase (MnSOD) and microsomal epoxide hydrolase (EPHX1), play a role in susceptibility to DA. The study population consisted of 252 workers exposed to diisocyanates (hexamethylene diisocyanate, methylene diphenyl diisocyanate, and toluene diisocyanate) of which 102 were diagnosed with DA based on a positive specific inhalation challenge. Genotype analysis was performed on genomic DNA, using a 5' nuclease PCR assay. The EPHX1 SNP (rs2740171) and GSTT1 gene deletion were associated with altered risk of developing DA after adjusting for potential confounders. Since EPHX1 and GSTT1 genes are important components of lung defense against oxidative stress, variations in these genes which regulate their expression may represent important disease modifiers and contribute to DA susceptibility.
This work was supported in part by an NIEHS IAG (Y1-ES-0001), NIOSH/CDC R01 OH 008795, and CDC Seed Funding for Public Health Genomics Research Program.

PS 1368 SULT1A1 GENE COPY NUMBER VARIATION (CNV) REAL-TIME QUANTITATIVE PCR (qPCR) ASSAY: AN OPPORTUNITY FOR POPULATION GENETIC COPY NUMBER VARIATION MEASUREMENT FOR ENHANCEMENT OF HUMAN HEALTH RISK ASSESSMENT OF GENETICALLY SENSITIVE SUB-POPULATIONS.

M. Young. *U.S. Environmental Protection Agency, Office of Science and Technology, Human Health Risk Assessment Branch, Washington, DC.*

Gene copy number variation (CNV) represents a novel source of heritable structural genetic variation in the human genome, and a potentially rich source of inter-individual phenotypic variation in the human population. Sulfotransferase 1A1 (SULT1A1) is a phase II xenobiotic metabolizing and detoxifying enzyme involved in the sulfonation of many different chemical and biochemical substrates. SULT1A1 single nucleotide polymorphism (SNP) variation in enzyme functional activity has implications for human population variation in toxicogenetics and pharmacogenetics, and SULT1A1 copy number variation has potentially comparable implications. A real-time quantitative polymerase chain reaction (qPCR) germline gene CNV laboratory assay was developed to determine the inherited gene copy number in human genomic DNA in the SULT1A1 gene. The assay employs fluorescent dual-labeled oligonucleotide hydrolysis probe chemistry and is capable of high-throughput large-scale analyses in laboratory and population studies. SULT1A1 germline gene copy number in a panel of human Coriell Cell Repository DNA samples was determined using the $2^{-\Delta\Delta CT}$ quantitative method, and demonstrates the application of the CNV assay. SULT1A1 germline gene copy number variation may represent a significant risk factor in gene-disease population association studies. SULT1A1 germline gene copy number variation may also be an important factor in modulating internal dosage of toxicological agents and modulating efficacy of pharmacological agents which are SULT1A1 chemical substrates.

[The opinions expressed herein are those of the author and do not necessarily reflect those of the U.S. Environmental Protection Agency.]

PS 1369 CHARACTERIZATION OF WHOLE GENOME AMPLIFIED (WGA) DNA FOR USE IN GENOTYPING ASSAY DEVELOPMENT.

T. Han[1], C. Chang[2], J. Kwekel[1], Y. Chen[3], F. Martinez-Murillo[4], D. Roscoe[4], Z. Tezak[4], R. Philip[4], K. Bijwaard[4] and J. C. Fuscoe[1]. *[1]Division of Systems Biology, National Center for Toxicological Research/FDA, Jefferson, AR, [2]DPNM/NCTR/FDA, Jefferson, AR, [3]DGMT/NCTR/FDA, Jefferson, AR and [4]ODDES/CDRH/FDA, Silver Spring, MD.*

Genome-wide genotyping assays often require substantial amounts of input DNA. To overcome the problem of limited clinical sample DNA, WGA methods have been developed. The multiple displacement amplification (MDA) method using phi29 polymerase has become the preferred choice because of its high processivity and low error rate. The uniformity of the amplification process across the genome has not been extensively characterized. In this study, we use array-based comparative genomic hybridization to evaluate DNA copy number variations (CNVs) produced in amplified DNA by two MDA kits: GenomiPhi (GE Healthcare) and Repli-G (Qiagen). The Agilent Human CGH array with 1 million features was used in this study together with DNA samples from a normal patient and two patients with cystic fibrosis. Each of the amplified DNA samples was compared to its native unamplified DNA with 4 independent replications. Hierarchical clustering analysis showed that there are significant differences between amplified and unamplified DNA samples with a median correlation of r=0.56. The correlations within unamplified (r=0.98) and amplified (r=0.96) groups, and the correlation between the amplification methods (r=0.94) were very high. Pair-wise testing was used to identify the probes that had CNVs changes. More than 20% of probes had fold changes greater than 2 after amplification compared to unamplified DNA samples. Application of a segmentation algorithm to reduce possibly spurious signals reduced to 15% the number of probes with more than 2-fold changes. Examination of the cystic fibrosis gene region showed no obvious CNV changes caused by the WGA process, suggesting that utility of the WGA DNA may depend on the particular use. These results indicate that challenges remain when WGA is applied for clinical use.

PS 1370 MUTAGENICITY OF RARE METAL AND METAL OXIDE NANOPARTICLES: COMPONENTS AND PARTICLE SIZES.

G. Hasegawa[1], M. Shimonaka[2] and Y. Ishihara[1]. *[1]Department of Public Health, School of Medicine, Kurume University, Kurume, Fukuoka, Japan and [2]Department of Chemistry, Tokyo University of Science, Tokyo, Japan.*

Nanoparticle of rare metals are used various products, however it is not enough to evaluate their carcinogenicity. Mutagenecity of four rare metals, indium oxide (In2O3), dysprosium oxide (Dy2O3), tungsten oxide (WO3) and molybdenum (Mo), that were defined particle diameter was demonstrated by in vitro bacterial reverse mutation assay (Ames test) using Salmonella thphimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP-2 uvrA strains. Size distribution of nano-sized rare metals in suspension was measured by dynamic light scattering photometer (DLS). All nano-sized rare metals were denoted as < 100 nm particle diameter (BET) by manufacturer, but the DLS results showed mean particle diameters for nano-sized In2O3, Dy2O3, WO3 and Mo, in suspension at 391.0 nm, 565.2 nm, 545.5 nm and 213.2 nm, respectively. Solubility of these metal compounds against neutral aqueous solutions was very low. Nano-sized Dy2O3 revealed strong mutagenic potential dose-dependently in all five bacteria strains tested with and without metabolic activation in Ames test, although micro-sized particles showed weak mutagenesis in two bacterial strains. Nano-sized In2O3 showed positive mutagenic response in TA1537, and nano-sized WO3 showed positive response in TA98 dose-dependently. While the micro-sized particle of these metal oxides showed no mutagenesis in the test bacterial strains. Both nano-sized and micro-sized Mo particles did not show any mutagenic effect. Although the mechanism of uptake the nano-sized metal compounds into the bacterial cells is unclear, size distribution and component of metal particles might influence their mutagenic effect. These results suggested that mutagenic effects of rare metals depend on their components and particle size, greater in nano-size than micro-size.
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PS 1371 PON1 Q192R POLYMORPHISM ANALYSIS IN SOUTHERN CARDIAC PATIENTS.

M. Dail, A. Crow, H. Coombes, E. Meek, H. Chambers and J. Chambers.
Center for Environmental Health Sciences, Mississippi State University, MS State, MS.

Paraoxonase (PON1) hydrolyzes paraoxon, the active metabolite of the insecticide parathion. The human PON1 gene has several single nucleotide polymorphisms (SNPs). The SNP involving a glutamine (Q) to arginine (R) substitution at codon 192 may be associated with the ability of PON1 to hydrolyze oxidized lipids, which protects against atherosclerosis. The question of whether the Q192R polymorphism is associated with cardiac disease is still open. Since southerners have the country's highest rate of cardiac disease, we collected blood and demographic data from 197 patients in a Mississippi cardiovascular clinic to compare the functional genotypes derived from serum PON1 hydrolytic activity to parameters of cardiac health. The multivariable model generated suggested that functional genotypic differences were insignificant compared to classic factors of atherosclerotic risk, but many of the patients were difficult to sort. To increase clarity, we sorted patients by their actual genotype and reexamined associations. Genomic DNA was isolated from blood and the 192 codon area was amplified by PCR. Since there is an AlwI restriction site at codon 192, the QQ, RR, and QR SNPs yield different digest patterns. The prior work found that Caucasians most often display the QQ functional genotype while African Americans have the RR functional genotype. This was confirmed. Other significant relationships: QQs are more often smokers whereas RRs are nonsmokers. QQs and RRs are more likely to have a family history of cardiac disease than QRs. HDL levels of RR patients were found to be significantly higher (P = 0.012) than those having a QQ genotype. RR patients were significantly younger (P = 0.02) than either QR or QQ patients. No significant relationships were seen between genotype and personal history of cardiac disease, statin use, or diabetes. No significant differences were seen among the three genotypes with regard to triglycerides, height, weight, waist size, LDL, or total cholesterol (Support NIHES015170)

PS 1372 ANALYSIS OF POLYMORPHISMS OF ASTHMATIC CHILDREN FROM COAHUILA, MEXICO EXPOSED TO ENVIRONMENTAL TOBACCO SMOKE (ETS).

B. Muñoz-Soto[1], B. S. Barrón-Vivanco[1], A. López-Moya[1], I. Romero-Toledo[1], C. López-Campos[2], J. J. Magaña-Aguirre[3] and A. Albores[1]. *[1]Toxicology, Cinvestav-IPN, México, Mexico, [2]Pediatrics, UMAE-IMSS 71, Torreon, Coahuila, Mexico and [3]Human Genetics, INR, Mexico City, Mexico.*

Recent studies argue that asthma prevalence in Mexico has increased 15% in the last 10 years; the constant exposure to allergens and environmental pollutants such as tobacco smoke is considered as one of the principal etiological factors. The vast majority of asthmatics are children; therefore, the aim of this study was to investigate the association between ETS exposure and some genetic polymorphism in asthmatic children. Our pilot study includes a population from Torreon, Coahuila, located in Northeast Mexico. Study groups included asthmatic children diagnosed according to ISAAC parameters (n=114) and a control group (n=100). These groups were subdivided in ETS-exposed children and non-exposed children, according to the parent's smoking habits. Spirometric studies and urine cotinine analysis were also performed. Real time PCR assays were used for genotyping IL13 (rs20541 and rs1800925), GSTP1 (rs1695), TNF (rs18000629) TSLP (rs1837253), ADBR2 (rs1042713) polymorphisms whereas conventional PCR was used for genotyping CYP1A1 (rs1048943). Our findings indicate that smoker's children showed higher cotinine levels than controls. On the other hand, we found no association between variants of IL13, GSTP1, TNF or CYP1A1 with asthma and exposure to ETS, however, other studies have shown this association. Remarkably, girls seems to be more susceptible to develop asthma (p=0.057); a strong association with TSLP (rs1837253) (p=0.0271) and marginal associations of ADBR2 (rs1042713) (p=0.099) with asthma was also observed. In addition, the TSLP mutated allele in asthmatic girls was also associated (p=0.072). This is the first study of asthma-related polymorphisms in Mexican children exposed to ETS.

PS 1373 *CYP1A1*2C*, *EPHX1T113H*, *EPHX1H139R*, AND *NQO1*2* POLYMORPHISMS ARE INVOLVED IN THE GENETIC DAMAGE CAUSED IN CHILDREN EXPOSED TO PAHS AND BENZENE.

M. Sánchez-Guerra[1], N. A. Pelallo-Martínez[2], F. Díaz-Barriga[2], L. Carrizales-Yáñez[2], L. Hernández-Cadena[3], M. E. Gonsebatt[4] and B. Quintanilla-Vega[1]. *[1]Toxicology Department, Cinvestav-IPN, Mexico City, Mexico, [2]Faculty of Medicine, UASLP, San Luis Potosí, Mexico, [3]National Institute of Public Health, Cuernavaca, Morelos, Mexico and [4]IIB, UNAM, Mexico City, Mexico.*

Polycyclic aromatic hydrocarbons (PAHs) and benzene are pollutants of urban cities and are considered genotoxic. Both compounds are metabolized and some enzymes such as CYP1A1 and CYP1B1 (activation) and NQO1 and GSTM (deactivation) and EPHX1 with both activities are polymorphic. We performed a cross-sectional study in children (6 to 10 years old, n=77) from primary schools located in the vicinities of the main industrial complex of Coatzacoalcos County, Veracruz, Mexico, an industrialized area with 80% of country's petrochemical activity, to evaluate the genetic damage of PAHs and benzene exploring the role of some metabolizing enzyme polymorphisms. Urinary 1-OHP levels and *trans*, *trans* muconic acid (*t,t* MA) as biomarkers of PAHs and benzene exposure, respectively, were determined by HPLC and *CYP1A1*2C*, *CYP1B1*3*, *EPHX1T113H*, *EPHX1H139R*, *NQO1*2* and *GSTM1*0* genetic polymorphisms by real time or conventional PCR. DNA damage was evaluated in lymphocytes by the Comet assay (tail moment). Thirteen percent of children had 1-OHP levels higher than 1.4 μmol/mol creatinine (NOAEL) and 32% had higher *t,t*-MA levels than the biological exposure index in the workplace (500 μg/g creatinine). DNA damage was significantly associated only with 1-OHP levels: children with 1-OHP levels above 1.4 μmol/mol creatinine showed a 23% increase of DNA damage, adjusted by *t,t*-MA (p=0.027). On the other hand, the DNA damage was higher in *CYP1A1*2C* homozygous children with 1-OHP levels above the NOAEL (54%, p=0.032); while children carrying one allele of *NQO1*2* or of *EPHX1H139R* polymorphisms had lower DNA damage (-37%, p=0.039 and -49%, p=0.007, respectively). These results suggest that polymorphisms of the studied metabolizing enzymes may have an important role in the development of adverse effects of urban environmental exposed children.

PS 1374 USING OF PCR-RFLP OF CYTOCHROME B GENE FOR HAIR IDENTIFICATION IN HUMAN AND SOME NON-DOMESTIC ANIMALS.

M. H. Ghoneim, A. A. Abou-Hadeed, A. M. Alkelch and M. R. Awad-allah.
Toxicology&Forensic Medicine, Zagazig University, Zagazig, Egypt.

PCR and PCR-RFLP methods were used to differentiate between human and some non domestic animals. DNA from hair was extracted to amplify a mitochondrial cytochrome b (cyt b) gene segment (358 bp) using universal primer followed by agarose gel electrophoresis. The total cyt b segment was digested with 4 restriction endonucleases,(Alu I, HaeIII, HinfI and Taq I) and the resulting fragments were resolved through electrophoresis. The different specific electrophoretic patterns and total restriction fragments were observed in each of the studied species. The restriction enzyme Alu I yielded 2 fragments of different sizes in all animal species. while no cleavage was observed in human hair. The digestion of PCR products with HaeIII restriction enzyme revealed restriction fragments patterns of different sizes in human and all species except in Bear. Using of HinfI restriction enzyme produced 2 or 3 fragments of different sizes for human and all species except Lama. When the amplified cyt b segment was digested by Taq I restriction enzyme it gave 2 bands of different sizes except Barbary sheep. There was variation in size of segments in human and different species.

PS 1375 STEM CELL REPONSES OF MURINE HIPPOCAMPUS FOLLOWING TRIMETHYLTIN INTOXICATION.

B. C. Weig, K. R. Reuhl and H. E. Lowndes. *Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Stem cells from the developing mammalian brain persist in adults in the subventricular zone (SVZ) and the hippocampal subgranular zone (SGZ). The response of SVZ and SGZ stem cells to hippocampal injury was studied in two-month old male C3H mice treated with trimethyltin (TMT). The fluorescent marker spDiI was injected unilaterally into the lateral ventricle of mice to label migrating SVZ cells. Bromodeoxyuridine (BrdU) was administered (80 mg/kg; ip) to label proliferating progenitor cells prior to treatment with TMT (2.7 mg/kg; ip). Sagittal brain sections taken 7 or 28d later revealed spDiI+ cells in the hippocampus of control mice at 7d, with a trend toward an increased number cells at 28d (p=0.0523). TMT injury had no effect on the number of BrdU+ or spDiI+ cells in the hippocampus at

7d. 28d after TMT injury there was a 4-fold increase in spDiI+ cells in the dentate gyrus granular and subgranular zone. Markers for astrocytes (GFAP) or neurons (NeuN) rarely co-localized suggesting the migrated spDiI+ cells remain in an undifferentiated state. TMT injury prevented the physiological loss of newly born (BrdU+) cells after 28d, either by increasing cell survival or recruitment of BrdU+ cells from the SVZ. Less than 15% of BrdU+ cells co-labeled with spDiI. To assess the role of cell proliferation in recovery from injury, mitosis was reduced by cranial exposure to 10gy gamma radiation 48hr prior to TMT injection, decreasing newly-born cells by 70%. TMT-induced tremors increased in severity and duration in irradiated mice, but tremors in all mice ceased by 5d post-TMT. TMT exposure caused prominent thrombomodulin staining in the parenchyma of the hippocampus dentate gyrus, indicating extravasation of blood-borne factors specifically at the site of injury. Data suggest that repair/recovery from TMT injury may include migration of SVZ cells into the injury site in addition to increased proliferation and survival of new neurons produced from endogenous hippocampal stem cells. Supported by ES011256, ES05022, and ES07148.

PS 1376 EFFECTS OF DIFFERENT FORMS OF COPPER ON HUMAN CNS DERIVED CELL LINES.

S. S. Shaligram and A. Campbell. *Pharmaceutical Sciences, Western University of Health Sciences, Pomona, CA.*

Copper is considered an essential metal for living organisms. However, excess copper is toxic owing to its ability to generate reactive oxygen species (ROS). This metal is directly linked to Menkes and Wilson's diseases. It is also implicated in age-related neurodegenerative disorders such as Alzheimer's disease. Copper is known to cause oxidative stress in the CNS. The purpose of this study was to study cellular response to soluble (copper sulfate) or insoluble (copper oxide) forms of this redox-active metal. We examined the formation of reactive oxygen species in human cell lines derived from the CNS after dosing them with different concentrations (0.5 μM- 625 μM) of copper sulfate or copper oxide. We assessed cell number and ROS formation after either 4 hr or 24 hr of copper exposure. In the SK-N-SH cells (neuronal), the number of cells decreased on exposure to the highest concentration of copper sulfate after 4 hr. After 24 hr exposure, the cell number increased at lower doses but decreased nonsignificantly at higher doses. With copper oxide, we saw a dose-dependent decrease in cell number after 4 hr and 24 hr. In A-172 cells (glioblastoma), the cell number decreased at higher doses with copper sulfate after 4 hr but showed no change at 24 hr. Exposure to copper oxide caused a dose-dependent decrease in cell number at both time points but the effect was more pronounced after 24 hr exposure. The neuronal cells showed a decrease in ROS formation after 24 hr with copper sulfate but showed no change after 4 hr, while the glioblastoma cells showed an increase in ROS formation at the higher concentrations of copper oxide after 24 hr exposure. Based on these results, we conclude that both forms of copper have some effect on the given cell lines but the response to copper oxide is more pronounced.

PS 1377 MECHANISM OF BRAIN COPPER (CU) TRANSPORT AND ACCUMULATION: INFLUENCE OF IRON DEFICIENCY.

A. D. Monnot[1], S. Ho[1], G. Robinson[2], Y. Pushkar[2] and W. Zheng[1]. [1]*Health Sciences, Purdue University, West Lafayette, IN and* [2]*Physics, Purdue University, West Lafayette, IN.*

Cu and iron (Fe) are essential for brain development and metabolism. Previous studies from this lab have shown an inverse physiologic relationship between Fe and Cu in the central nervous system. Despite its critical role in normal brain function, knowledge on Cu transport, distribution, homeostatic regulation, and the impact of Fe status, remains largely unexplored. This study was designed to investigate the regulation of Cu transport by Fe status at the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCB), as well as the distribution of Cu in the brain. Rats were divided into 3 groups with special diets at libertum for 4 weeks: control (35 mg Fe/kg), Fe deficient (FeD) (3-5 mg Fe/kg), and Fe overload (FeO) (20 g carbonyl Fe/kg) groups. Serum Fe status, either FeD or FeO, was confirmed by total Fe, unsaturated Fe binding capacity, total Fe binding capacity, and transferrin saturation in serum. In situ brain perfusion of 64Cu demonstrated that the rate of Cu transport was significantly faster in brain parenchyma (+92%) and capillaries (+500%) in FeD rats than those in controls. In contrast, FeO treatment significantly reduced the rate of 64Cu transported into brain parenchyma (~50% of controls). Additionally, Cu clearance from the CSF was greater in FeD than in control animals as demonstrated by ventriculo-cisternal brain perfusion, suggesting an increased Cu uptake from the CSF by the BCB. Results from mRNA and protein expression studies indicated that FeD was responsible for the up-regulation of DMT1, but not CTR1, at both the BBB and BCB, which may contribute to the enhanced Cu uptake found at the barriers. X-ray fluorescence imaging indicated Cu accumulation in the subventricular zone (SVZ) of the lateral ventricles. An elevated Cu level (+25%), although not statistically significant, was found in the SVZ of FeD. These results suggest that DMT1 regulation at the brain barriers, but not at SVZ, contribute to Cu accumulation and regulation during FeD in the brain. (Supported by NIH/RO1-ES008146)

PS 1378 PERSISTING NEUROCHEMICAL EFFECTS OF DEVELOPMENTAL COPPER EXPOSURE IN WILDTYPE AND METALLOTHIONEIN 1 AND 2 KNOCKOUT MICE.

A. Petro[1], H. Sexton[1], C. Miranda[1], A. Rastogi[1], J. H. Freedman[3] and E. D. Levin[1, 2]. [1]*Psychiatry, Duke University Medical Center, Durham, NC,* [2]*Integrated Toxicology and Environmental Health Program, Duke University, Durham, NC and* [3]*NIEHS, Research Triangle Park, NC.*

Metallothioneins are small proteins, which are crucial for the distribution of heavy and transition metals. This series of studies was conducted to determine the role of metallothioneins on metal-induced neurotoxicity during development. Previously, we found that knockout of MT 1 and 2 potentiated the cognitive impairment caused by developmental mercury exposure. The current study examined the neurocognitive effects of developmental copper supplementation. Wildtype and MT 1 and 2 knockout mice (MTKO) were given 10 or 50 mg/l of supplemental copper during gestation and until weaning. Mice of both genotypes with no added copper served as controls. When the mice were young adults they were trained for 18 sessions on the win-shift 8-arm radial maze test of spatial learning and memory. Male MTKO mice with no added copper were significantly impaired on radial-arm maze choice accuracy relative to wildtype controls. This MTKO-induced impairment was significantly attenuated by addition of 10 mg/l but not 50 mg/l of copper supplementation during development. Neurochemical analyses showed that MTKO caused a significant overall increase in serotonin in all of the regions studied: the frontal cortex, posterior cortex, hippocampus, striatum, midbrain, and brainstem. MTKO also caused a significant increase in norepinepherine in the brainstem and hippocampus. In wildtype mice, copper supplementation during development caused a significant decline in dopamine and norepinepherine levels in the midbrain and dopamine in the frontal cortex. These effects were blocked by knockout of MT 1 and 2.
(Supported by NIH ES10356)

PS 1379 PERSISTENT OLFACTORY DEFICITS FOLLOWING INTRANASAL CADMIUM EXPOSURE CAN BE REHABILITATED BY TRAINING ON AN OLFACTORY DETECTION TASK.

D. J. Turkel, A. H. Moberly, L. Czarnecki and J. P. McGann. *Psychology Department, Rutgers University, Piscataway, NJ.*

We have previously shown that acute intranasal exposure to cadmium disrupts olfactory nerve function, including a large decrease in odorant-evoked neurotransmitter release, reduced innervation of the olfactory bulb, and profound impairment on an olfactory detection task in the mouse. These deficits persist for at least four weeks. However, some olfactory nerve function remains intact following cadmium exposure, which could in principle be sufficient to detect an odorant. We hypothesized that post-exposure olfactory training could potentially rehabilitate detection performance. C57Bl/6 mice were trained to perform an odorant-guided go/no-go odor detection task in which they sampled at an odor-port and then received a sucrose reward for a nose poke in a dipper port if and only if the odorant was presented. Incorrect responses were punished by a lengthened intertrial interval. After mice achieved criterion performance, they were anesthetized and received bilateral intranasal instillations of either 20 μg cadmium/side or vehicle control. They were randomly assigned to either a 2-day delay or 10-day delay group. Cadmium-exposed mice tested at either time point were profoundly impaired on the detection task, with an average d' of about 0.15 in both groups. After testing, the worst performing mice from the 2-day group were trained daily on the discrimination task until they again achieved criterion performance. Remarkably, by Day 10 this group's performance was not only significantly better than that of the untrained group at Day 10, but comparable to baseline performance. Rehabilitated mice tested without odorants exhibited no discrimination, confirming that they were indeed using their damaged olfactory systems to perform the task. This recovery of detection ability suggests that downstream brain regions can successfully learn to interpret olfactory degraded sensory input. In addition to informing potential therapies, these data suggest that rapid sensory learning could potentially mask the effects of serious pathology.

PS 1380 *IN VIVO* OPTICAL VISUALIZATION OF DOSE-DEPENDENT PATHOPHYSIOLOGY INDUCED BY INTRANASAL CADMIUM EXPOSURE IN THE MOUSE OLFACTORY SYSTEM.

L. Czarnecki, A. H. Moberly, J. Pottackal, D. J. Turkel, T. Rubinstein and J. P. McGann. *Psychology Department, Rutgers University, Piscataway, NJ.*

Intranasal exposure to cadmium has been related to olfactory dysfunction in humans and to nasal epithelial damage and altered odorant-guided behavior in rodent models. The pathophysiology, neural pathology, and perceptual deficits underlying these changes have not been fully elucidated. Transgenic mice expressing the fluorescent exocytosis indicator synatopHluorin (spH) in olfactory receptor neuron terminals (Bozza et al. 2004, Neuron, 9-21) received a unilateral intranasal instillation of 0.2 to 20 μg cadmium chloride and a contralateral vehicle instillation. Mass spectrometry demonstrated transport of this cadmium to the ipsilateral olfactory bulb. Mice were anesthetized, implanted with a cranial window overlying the olfactory bulbs, and odorant-evoked transmitter release from the olfactory nerve was quantified bilaterally via optical imaging of spH signals. Acute intranasal cadmium exposure reduces this odorant-evoked response from the olfactory nerve by up to 65% in a dose-dependent manner. Axonal projections from the olfactory epithelium to the olfactory bulbs were quantified in horizontal sections containing both bulbs. Cadmium exposure reduced the density of these projections by 20% at the highest dose of cadmium, consistent with histologically identified changes in the olfactory epithelium. In a behavioral psychophysical task, mice were trained to sample from an odor port and make a response when they detected an odorant against a background of room air. After bilateral intranasal cadmium instillations, mice were severely impaired in their ability to detect the target odor. This use of functional imaging of olfactory receptor neurons exposed to the toxicant serves a proof of concept that optical imaging in the olfactory system can permit the direct observation of exposure-induced pathophysiology in vivo. This approach is potentially useful in exploring the effects of any putative neurotoxicant that can be delivered intranasally.

PS 1381 PERIPHERAL CADMIUM EXPOSURE INDUCES SECONDARY PATHOLOGY IN DOWNSTREAM BRAIN REGIONS IN THE MOUSE OLFACTORY SYSTEM.

J. Pottackal, D. J. Turkel, T. Rubinstein, M. D. Kass, L. Czarnecki and J. P. McGann. *Psychology Department, Rutgers University, Piscataway, NJ.*

The mammalian olfactory system is highly plastic, including central olfactory structures. This plasticity may be important to understanding the effects of inhaled toxicants because pathological changes in neurophysiology or neurochemistry of the brain may occur indirectly in response to a toxicant's primary effects. We have previously demonstrated that acute intranasal instillation of cadmium chloride causes a profound reduction in odorant-evoked transmitter release from the olfactory nerve into the brain's olfactory bulb, a deficit that persists for at least four weeks. Here we used immunohistochemical methods to document corresponding changes in the neurochemistry of olfactory bulb neurons that receive synaptic input from the olfactory nerve. Mice received unilateral intranasal instillations of 20 μg cadmium chloride and were sacrificed by intracardial perfusion 2 to 28 days later. Horizontal sections containing both olfactory bulbs were labeled by fluorescence immunohistochemistry for tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine production, or calbindin-d28k (CBD), an intracellular calcium-binding protein, and counterstained with DAPI. At two weeks post-exposure, but not earlier time points, periglomerular interneurons in the olfactory bulb exhibited a significant reduction in TH expression in sections from bulbs on the cadmium-exposed side compared to the contralateral bulb in the same section. This downregulation became more pronounced with time, reaching 50% by the 28 day time point. Unilateral naris occlusion mimicked this effect of cadmium-exposure, suggesting that the downregulation was caused by the reduction in sensory input rather than by cadmium intoxication per se. CBD levels were slightly elevated from 14-28 days, which could not be mimicked by naris occlusion. These data demonstrate that peripheral toxicant exposure can produce long-term secondary pathologies in downstream brain regions. Mere reversal of the peripheral damage may thus be insufficient to restore normal neural function.

PS 1382 FAILURE TO REGENERATE OLFACTORY NERVE FUNCTION AFTER ACUTE INTRANASAL CADMIUM EXPOSURE IN THE MOUSE.

T. Rubinstein, L. Czarnecki, D. J. Turkel, J. Pottackal and J. P. McGann. *Psychology Department, Rutgers University, Piscataway, NJ.*

The mammalian olfactory epithelium undergoes a constant cycle of cell death and neurogenesis, such that the life span of a single olfactory receptor neuron is about four weeks. Here we report that olfactory pathophysiology following acute intranasal cadmium exposure does not recover over the succeeding month, as would be expected, but actually worsens over time. Transgenic mice expressing the fluorescent exocytosis indicator synatopHluorin (spH) in olfactory receptor neuron terminals (Bozza et al. 2004, Neuron, 9-21) received a unilateral intranasal instillation of 20 μg cadmium chloride and a contralateral vehicle instillation. After 2 to 28 days, mice were anesthetized, implanted with a cranial window overlying the olfactory bulbs, and odorant-evoked transmitter release from the olfactory nerve was quantified bilaterally via optical imaging of spH signals. Odorant-evoked release from the olfactory nerve on the cadmium-exposed side declined significantly over time, from approximately 35% of control two days after exposure to about 10% of control over the succeeding four weeks. Axonal projections from the olfactory epithelium to the olfactory bulb were quantified by measuring the optical density of spH expression (serving as an indicator of olfactory marker protein expression) in horizontal sections containing both olfactory bulbs. This labeling was reduced to 80% of control levels two days after cadmium exposure, and then declined significantly to about 40% of control over the succeeding four weeks. The persistence of olfactory nerve pathophysiology following intranasal cadmium exposure is unexpected in this regenerating system, and the steady deterioration of the neuronal responses is consistent with a failure to replace receptor neurons as they turn over. This suggests that cadmium may impair neuronal proliferation in the nasal epithelium.

PS 1383 THE EFFECT OF TUNGSTEN ALLOY SURROGATES ON PC12 CELL GENE EXPRESSION.

V. H. Adams[1], D. I. Bannon[1], M. G. Stockelman[2] and V. P. Mokashi[2]. [1]*Army Institute of Public Health, U.S. Army Public Health Command (Prov), Aberdeen Proving Ground-EA, MD and* [2]*Naval Medical Research Unit, Dayton (NAMRU-Dayton), Wright-Patterson Air Force Base, OH.*

The neurotoxicity of metals such as tungsten (W), nickel (Ni), and cobalt (Co) has not been examined in detail. Moreover, the combined effect (whether additive, synergistic or neither) of these metals in a mixture is even less understood. Prior data indicate that deposition of radiolabeled tungstate ($Na_2{}^{188}WO_4$) in the brain is not accomplished through direct transport mechanisms along the olfactory nerves; however, investigators have reported that tungsten can be detected in rat brain following intravenous or intraperitoneal injection and oral gavage. As an alternative to *in vivo* testing, we used the PC12 cell *in vitro* model to explore the transcriptional profile of cells exposed to a tungsten alloy containing W, Ni and Co. Since W alloy becomes bioavailable during exposure, the ionic forms of the metals (WO_4^-, Ni^{+2}, Co^{+2}) were used as an alloy surrogate. Based on Inductively Coupled Plasma – Mass Spectrometry analysis of tungsten alloy pellets incubated in PC12 cell tissue culture medium, the ratio of 3:2:1 mg/L (equivalent to 16, 34, and 17 uM respectively) WO_4^-, Ni^{+2}, Co^{+2} was selected for the PC12 cell exposure condition. In addition to testing the complete alloy the individual metal ions and metal ion combinations (W/Ni, W/Co, Co/Ni) were also tested at the indicated concentrations. PC12 cells were incubated in the presence of neuronal growth factor and the metal ions (or without metals as a negative control) for 24 hours followed by RNA extraction. Each test condition was performed in triplicate wells and the entire experiment was repeated for a total of 4 replicates. For the microarray analysis, Affymetrix Rat Genome 230 2.0 whole genome oligonucleotide arrays were used. Expression data was analyzed using JMP Genomics 4.1 (SAS) software. Our preliminary findings suggest that there are differences between the gene expression profiles of the single metal and alloy treatments. The changes in gene expression as it relates to the presence of tungstate will be discussed.

PS 1384 DIFFERENT SUSCEPTIBILITIES TO MEHG-INDUCED CELL DEATH BETWEEN NON-NEURONAL AND NEURONAL CELLS.

V. Vidal[1], S. M. Fox[2] and W. D. Atchison[2]. [1]*University of Puerto Rico at Cayey, Cayey, Puerto Rico and* [2]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Methylmercury (MeHg) is a highly lipophilic environmental neurotoxicant that causes selective damage in both the central and peripheral nervous system. Among the many cellular targets associated with the effects of MeHg are voltage-gated Ca^{2+} channels. The action of MeHg is identified to be a direct interaction with the Ca^{2+} channels subunits as well as an interaction with intracellular signaling pathways that modulate their activity. The goals of this project were to determine the difference in susceptibility to MeHg-induced cell death between undifferentiated PC12 cells, a neuronal cell line, and human embryonic kidney (HEK) cells, a non-neuronal cell line, as well as, to study the contribution of the L-type Ca^{2+} channel (Cav 1.2) to MeHg-induced cell death. PC12 cells express Cav1.2 abundantly- especially in the undifferentiated state; HEK cells do not. HEK cells were transiently transfected with Cav 1.2 (HEK-α1C) in order to assess the contribution of the L-type Ca^{2+} channel in the absence of other neuronal targets of MeHg. The transfection utilized expression cDNA plasmids containing α1C, β3, and α2δ Ca^{2+} channel

subunits. Each cell type was treated with 0.5, 1, 2, or 5 μM MeHg for 2 h and cell viability was measured 24 h later using trypan blue. Concentration- dependence of cytotoxicity was seen in each cell type. PC12 cells had a greater reduction in cell viability than both HEK and HEK-α1C after treatment with MeHg. At 0.5 and 1 μM MeHg there was little change in viability in either cell type. At 2 μM MeHg cell viability in both HEK and HEK-α1C cells was ~97% while it was 88% in PC12 cells. Cell viability was 85%, 90% and 80% in HEK, HEK-α1C, and PC12 cells, respectively, after treatment with 5 μM MeHg. Using this model system neuronal cells are more susceptible to MeHg-induced cell death than non-neuronal cells. Supported by 5R01-ES03299 and 5R25NS065777.

PS 1385 EFFECTS OF ACUTE EXPOSURE TO METHYLMERCURY ON INTRACELLULAR CA^{2+} IN PRIMARY CEREBELLAR GRANULE CELLS OF *TOTTERING* AND *LETHARGIC* MICE.

B. Marrero-Rosado[1] and W. D. Atchison[2]. [1]*Genetics Program, Michigan State University, East Lansing, MI and* [2]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Methylmercury (MeHg), preferentially affects cerebellar granule cell (CGC) function both *in vivo* and *in vitro*. In CGCs MeHg disrupts intracellular Ca^{2+} (Ca^{2+}_i) in a biphasic manner involving distinct effects on Ca^{2+}_i stores, and extracellular entry. CGCs contain several types of voltage-gated Ca^{2+} channels (VGCCs), all of which are susceptible to block by MeHg, but also contribute to the increased $[Ca^{2+}]_i$ in CGCs. The possibility that certain VGCC subtypes may facilitate effects of MeHg in native cells has not been examined. In vitro acute exposure to MeHg and single cell imaging was used to examine $[Ca^{2+}]_i$ homeostasis in CGCs of two naturally-occurring mutants of the P/Q-type VGCC (*tottering-tg* and *lethargic-lh*) mice. *Tg* results from a spontaneous missense mutation in the gene *Cacna1a* that codes the pore-forming α_{1A} subunit of P/Q-type VGCCs. To compensate, *tg* CGCs upregulate N-type VGCCs. *Lh* mice have a 4-base pair insertion in the β_4 subunit gene, *Cacnb4,* truncating the subunit, preventing binding to α_{1A}. *Lh* mice compensate by upregulating β_{1B} and β_3. CGCs were isolated at PND 7, loaded with Fura-2 and acutely exposed to 0.3, 1, or 3 μM MeHg. Onset times of the 2 phases of increased $[Ca^{2+}]_i$ were compared among genotypes. *Tg* CGCs do not differ significantly from WT for onset of either phase of MeHg-induced increased $[Ca^{2+}]_i$, but *lh* CGCs reach both phases more rapidly than their WT littermates. To investigate whether adult *tg* CGCs would respond differently to MeHg, cells were artificially matured by lowering medium $[K^+]$. Both phases of $[Ca^{2+}]_i$ increase were now significantly delayed in onset. This raises the possibility of a gene/environment contribution to the effects of MeHg in cerebellum. Supported by R01ES03299, and R25NS065777.

PS 1386 METHYLMERCURY DECREASES NEURONAL MIGRATION, VINCULIN LEVELS, AND LOCATION IN FOCAL ADHESION OF A NEUROBLASTOMA CELL LINE.

D. Albores-Garcia[1], L. C. Acosta-Saavedra[1], E. K. Silbergeld[2] and E. S. Calderon-Aranda[1]. [1]*Department Toxicologia, Cinvestav, Mexico, Mexico and* [2]*BSPH, Johns Hopkins University, Baltimore, MD.*

Methylmercury (MeHg) is an organometal that inhibits neuronal migration during the central nervous system development, and for which the molecular mechanisms are not completely defined. The neuronal migration involved the assembly and disassembly of focal adhesions (FA), conformed by a complex of proteins that include vinculin, paxillin and FAK (focal adhesion kinase), as well as kinases like MAPKs (mitogen associated protein kinases). Our group has shown in primary cultures of mouse cerebellum, that MeHg decreases the phosphorylation of ERK, p38 and JNK, as well as paxillin levels, effects that were related with inhibition of neurons migration. The objective of this work was to study the effect of MeHg on neuronal migration, vinculin levels, and vinculin location in focal adhesion in a human neuroblastoma cell line. Methods. Human neuroblastoma cell line SH-SY5Y activated or non activated to migration with 50ng/mL PDGF (platelet-derived growth factor), were treated with 100nM of MeHg, and cell migration was evaluated by Scratch method, vinculin levels by Western Blot and vinculin location in FA (determined as vinculin-FAK co-location) was by confocal microscopy. Results showed that MeHg decreases migration induced by PDGF at 6 h of treatment, as well basal migration in non activated cells. MeHg, both in absence and presence of PDGF, decreased the vinculin levels compared with their respective controls. MeHg decrease size of adhesion points corresponding to vinculin and FAK, and its co-location, compared with non activated control; in activated cells MeHg decreased the number and size of FA with vinculin, compared with levels of vinculin in FA adhesion induced by PDGF. These results suggest that inhibition of neuronal migration by MeHg involves the alteration of focal adhesions, and that further study is required to establish the effect of MeHg on molecules that are involved and/or regulated the assembly and disassembly of FA adhesions. This work was supported by Conacyt-46297.

PS 1387 EFFECT OF METHYLMERCURY ON THE NEURONAL MIGRATION AND ON ACTIVITY OF FAK IN HUMAN NEUROBLASTOMA CELLS.

G. T. Rodriguez-Kessler[1], D. Albores-Garcia[1], L. C. Acosta-Saavedra[1], B. Luque[1], D. Arias-Salvatierra[1], E. K. Silbergeld[2] and E. S. Calderon-Aranda[1]. [1]*Toxicologia, Cinvestav, Mexico, Mexico and* [2]*BSPH, Johns Hopkins University, Baltimore, MD.*

Methylmercury (MeHg)inhibits neuronal migration but molecular mechanisms are poorly understood. Neuronal migration is a multistep process with rapid changes that involved assembly and disassembly of focal adhesions (FA). Focal adhesion kinase (FAK) is an essential component of the FA that regulates the recruitment and activation of other proteins. ERK and JNK (MAPKs)regulate interactions of FAK with other proteins like paxillin (Pax), and the assembly and disassembly of FA. We have shown that exposure to MeHg decreases the phosphorylation of MAPKs, the levels of Pax and the neuronal migration in cerebellar primary cultures; we established that MeHg reduces the levels of vinculin, another key element of FA, and inhibits neuronal migration of the human neuroblastoma cells. Although these data partly explain the mechanism by which MeHg inhibits neuronal migration, it is necessary to evaluate the effect of MeHg on critical proteins involved in regulation of AF. The objective in this work is to study the effect of MeHg on neuronal migration, the phosphorylation of critical residues for the activation of FAK and for its interaction with Pax, the role of ERK and JNK in these effects, as well as the relationship of all these. Methods. In SH-SY5Y cells activated or not to migration with PDGF (50ng/mL) the effect of 100ng/mL of MeHg is assess on cell migration by scratch assay, phosphorylation of FAK is by Western-blot, and role of ERK and JNK in these effects is evaluated using specific inhibitors.Results shown that MeHg decreases migration induced by PDGF,as well as the basal migration in non activated cells.With inhibitors of ERK and JNK was determined that both kinases are involved in cell migration induced by PDGF.Experiments to evaluate the MeHg effect on FAK and Pax phosphorylation are in progress. This work was supported by Conacyt-46297.

PS 1388 DIFFERENTIAL EFFECTS OF NEUROTOXIC MERCURIALS ON ANGIOGENESIS (AG).

M. Nash[1], E. Robinson[1], B. J. Zaffo[1], S. Obhan[2], N. F. Alrowaily[1], L. A. Shaiba[1], S. A. Mousa[2] and H. A. El-Fawal[1]. [1]*Health Sciences, Albany College of Pharmacy and Health Sciences, Albany, NY and* [2]*Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Albany, NY.*

The elaboration of vascular beds, AG, is essential during neurogenesis. Compromised neural AG in adult models has been shown to result in neuropathy and neurodegeneration. Mercurial compounds (Hg), organic [methy (MeHg) and ethyl (EHg)] and inorganic [mercuric chloride (HgCl)] are known developmental neurotxicants. However, their effects on AG as a contributing factor in inducing neurotoxicity have not been investigated. The effects of equimolar concentrations (40 nM) of these compounds on AG, in the presence or absence of 40 μg vascular-endothelium growth factor (VEGF) were investigated in the 10 day chick chorioallontoic membrane (CAM) model. Test compounds placed on coverslips were added onto the membrane of CAM and harvested after 3 days of incubation under optimal condition of O_2/CO_2 and humidity to visualize and quantify developing vessel length (L), number of branch points (B) and perfusion area (A). All Hg compounds reduced B by 40-60% with the greatest reduction seen with HgCl. Significant ($p<0.05$) decreases in L and increases in A were measured for EHg and HgCl. In contrast, MeHg treatment significantly increased L and decreased A. For all treatments, except MeHg on B, effects were partially reversed in the presence of VEGF. The reduction in B, with an increase in L and decreased A, suggests a failed attempt at compensation, but would fail to provide adequate perfusion in absence of anastomoses. Taken together, this study demonstrates that neurotoxic Hg compounds may interfere with AG during development. In the context of neurodevelopment, as is currently being investigated, this may potentially contribute to the morphological and functional deficits observed as a result of congenital and early post-natal exposures.

PS 1389 ANTAGONISTIC EFFECTS OF NEUROTOXIC MERCURIALS ON TUMOR-INDUCED ANGIOGENESIS (AG).

B. J. Zaffo[1], M. Yalcin[2], E. Robinson[1], L. A. Shaiba[1], N. F. Alrowaily[1], S. A. Mousa[2] and H. A. El-Fawal[1]. [1]*Health Sciences, Albany College of Pharmacy and Health Sciences, Albany, NY and* [2]*Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Albany, NY.*

Developmental neurotoxicity of mercury (Hg) has long been recognized. A potential contributing factor may be the inhibition of AG. This was investigated for methyl (MeHg), ethyl (EHg) and mercuric chloride (HgCl) in the 10 day chick

chorioallontoic membrane (CAM) and in the mouse matrigel models. Equimolar concentrations (40 nM) of test compounds were combined with the basement membrane matrix matrigel seeded with 10^6 pancreatic tumor cells (PANC-1), incubated with CAM and harvested after 7 days to determine tumor mass and hemoglobin (Hb) concentrations (mg/ml) as indices of AG. PANC-1 cells are an established source of numerous angiogenic factors, including vascular-endothelium growth factor and fibroblast growth factor (FGF-2). All Hg compounds significantly ($p<0.05$) reduced tumor mass with the greatest decrease seen in the presence of MeHg. This pattern was mirrored by the reduction in Hb which was 50-75% of FGF controls. However, MeHg- and HgCl-induced Hb decreases were similar. Together these results demonstrate a differential effect of Hg compounds on AG of: EHg < MeHg < HgCl. This is consistent with inorganic Hg compounds having significant peripheral vascular effects. These results were confirmed by subcutaneous injection of matrigel containing 1 μg/plug of Hg compounds in the presence or absence of FGF-2 (200ng/plug) in male C57BL/6NCr mouse thigh (n=16). Concentrations of Hb were determined as an index of growth factor-induced AG and its modulation by Hg compounds. All Hg compounds were equally effective in inducing a significant ($p<0.05$) reduction in basal Hb concentrations. This was only partially prevented by FGF-2. The results confirm that Hg compounds interfere with AG. Investigations of the mechanisms involved, cytotoxic or physiological, and the potential neuroteratogenicity is underway.

PS 1390 METHYLMERCURY AFFECTS HIPPOCAMPAL NEURAL STEM CELLS LEADING TO LATER CELL LOSS AND MEMORY DEFICITS.

K. B. Sokolowski[1, 3, 4], K. Robinson[2] and E. DiCicco-Bloom[4, 3, 1]. [1]*JGPT, Rutgers, New Brunswick, NJ,* [2]*CBN, Rutgers, New Brunswick, NJ,* [3]*GSBS, UMDNJ, Piscataway, NJ and* [4]*NCB, RWJMS, Piscataway, NJ.*

MeHg, an environmentally persistent teratogen, induces a spectrum of harmful effects on brain structure and function. Our recent studies indicated that a single sc injection of 5μg/gbw MeHg in 7 day old (P7) rat pups acutely activates caspase-3, an apoptotic protease, leading to cell cycle arrest and cell death specifically in hippocampus and not cerebellum. Caspase-3 immunoreactivity was therefore used to define lower exposures of MeHg that induce significant increases in hippocampal cell death. We found a five-fold increase in caspase-3+ cells 24h after 0.6μg/gbw MeHg, a ten-fold lower exposure. Also, BrdU labeled mitotic cells were decreased by ~30% of the control in proliferative regions of hippocampus (32% hilus (H), 27% granule cell layer (GCL)). Double labeling revealed that ~80% of caspase-3+ cells were colabeled with nestin and ~35% with Sox2, both neuronal stem cell markers. In contrast, GFAP staining in hilus was unchanged with 0.6μg/g MeHg compared to controls, although an increase was detected at 5μg/gbw MeHg. The apparent selective sensitivity of neuronal stem cells in hippocampus at this low exposure correlated with a 22% and 27% decrease in total cells in H and GCL, but no change in nonproliferative regions, two weeks after MeHg (P21) defined by stereology. In addition, there was a subtle learning deficit coupled with a significant increase in escape latency in memory trials assessed by Morris water maze (2-day memory: Con: 18±3sec, MeHg: 28±4sec, $p=0.03$). Together, these results illustrate that a single injection of 0.6μg/g MeHg at P7 selectively targets neuronal stem cells for apoptosis that may lead to decreases in neurons in the dentate gyrus two weeks later at P21 and memory deficits during adolescence at P35.

PS 1391 PRENATAL EXPOSURE TO METHYLMERCURY NEGATIVELY AFFECTS BDNF CONCENTRATION IN CORD BLOOD.

S. Spulber[1], T. Rantamäki[2], O. Nikkilä[2], E. Castrén[2], P. Weihe[3], P. Grandjean[4, 5] and S. Ceccatelli[1]. [1]*Neuroscience, Karolinska Institutet, Stockholm, Sweden,* [2]*Neuroscience Center, University of Helsinki, Helsinki, Finland,* [3]*Faroese Hospital System, Tørshavn, Faroe islands,* [4]*Institute of Public Health, University of Southern Denmark, Odense, Denmark and* [5]*Environmental Health, Harvard School of Public Health, Boston, MA.*

Brain-derived neurotrophic factor (BDNF) is a neurotrophin essential for neuronal survival and differentiation. We examined the concentration of BDNF in cord blood samples from newborn exposed to methylmercury (MeHg) in utero by maternal consumption of whale meat. The cohort consisted of 395 singleton births (206 boys; 189 girls), gestational age ranging from 38 to 42 weeks. BDNF was measured by sandwich ELISA run on serum from cord blood samples collected in connection with parturition. Maternal smoking habits and other relevant factors were estimated by interviewing the mothers. The exposure to MeHg was estimated based on Hg measured in the cord blood. Prenatal MeHg exposure affected serum-BDNF, which was slightly decreased in a concentration-dependent manner in girls born to non-smoking mothers. Maternal smoking significantly increased BNDF in girls, but not in boys. For further statistical analyses, we used serum-BDNF concentration as continuous outcome variable in supervised regression models. According to our model, serum-BDNF concentration increases with gestational age; is increased by maternal smoking; is slightly decreased by MeHg exposure; and maternal smoking enhances the decrease in serum-BDNF induced by MeHg exposure. Cord blood-BDNF has been reported to increase in association with perinatal injuries and proposed as possible predictive marker of more favourable neurodevelopmental outcome. The negative effect that MeHg seems to exert on cord blood-BDNF concentration may thus endanger the compensatory response to an adverse milieu with latent detrimental consequences for the developing nervous system.

PS 1392 A ROLE FOR CYTOCHROME P450 (CYP) ENZYMES IN METHYLMERCURY (MEHG) TOLERANCE.

M. D. Rand and C. Mahapatra. *Anatomy and Neurobiology, University of Vermont, Burlington, VT.*

Methylmercury (MeHg) is a ubiquitous environmental toxin that preferentially targets the developing nervous system. Variable outcomes of prenatal MeHg exposure among different populations, and among individuals within a population, point to a genetic component that regulates MeHg toxicity. We have employed the Drosophila model in an unbiased screen for genes that confer tolerance to MeHg during development. We identified cytochrome p450 (CYP) gene family members as potential MeHg tolerance genes with CYP6g1 being the most highly expressed and regulated CYP in response to MeHg in lab-selected tolerant flies. We now demonstrate that transgenic expression of CYP6g1 confers a significant degree of MeHg tolerance in developing flies. Serendipitously, a single high-expressing allele of CYP6g1 in wild-derived flies has been elucidated as the DDT resistance allele (DDT-R) that has spread globally in Drosophila. We demonstrate a robust MeHg tolerance in a fly strain carrying the DDT-R allele. Additional wild derived flies lines exhibiting elevated CYP6g1 expression show high levels of MeHg tolerance. The CYP genes are noted for their large numbers and diversity across the Phyla (e.g. 57 human CYPs and 83 Drosophila CYPs). BLAST (NCBI) searches identify the highest identity and similarity scores between CYP6g1 and human CYP3A4, CYP3A5 and CYP3A7. The CYP3A family members are the most highly expressed liver CYPs and the predominant enzymes for xenobiotic metabolism. An intriguing correlation exists between high activity polymorphisms of CYP3A7, CYP3A5 and CYP3A4 and the apparent absence of neurological outcomes in MeHg exposed children of the Seychelles Childhood Developmental Study. These observations have prompted investigation of the human CYP3A family genes as MeHg tolerance/susceptibility markers.

PS 1393 OXIDATIVE STRESS-MEDIATED ERK1/2, P38 AND MITOCHONDRIAL-DEPENDENT SIGNALING PATHWAYS INVOLVE IN METHYLMERCURY INDUCES THE NEURONAL CELL APOPTOSIS.

C. Chen[1, 2], T. Lu[2], C. Yen[3], D. Hung[4, 2], T. Ho[5], C. Su[6], Y. Chen[7], C. Huang[5] and W. Chen[1]. [1]*Emergency Department, China Medical University Hospital, Taichung, Taiwan,* [2]*Graduate Institute of Drug Safety, China Medical University, Taichung, Taiwan,* [3]*Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, Taiwan,* [4]*Toxicology Center, China Medical University Hospital, Taichung, Taiwan,* [5]*School of Chinese Medicine, China Medical University, Taichung, Taiwan,* [6]*Department of Otorhinolaryngology, Head and Neck Surgery, Changhua Christian Hospital, Changhua, Taiwan and* [7]*Department of Physiology, China Medical University, Taichung, Taiwan.*

Methylmercury(MeHg) is a well-known highly toxic metal that causes severe irreversible damage in central nervous system and the risk factor for inducing neuronal degeneration. The molecular mechanisms of MeHg-induced neurotoxity remain unclear. We investigated the toxic effects and possible mechanisms of MeHg on Neuro-2a cells. Treatment of Neuro-2a cells with MeHg was significantly reduced cell viability, increased ROS formation, and depleted intracellular glutathione. MeHg also induced several features of mitochondria-dependent apoptotic signals including increase of sub-G1 hypodiploid cells, disruption of mitochondrial membrane potential, increase of mitochondrial cytochrome c release, up-regulation of Bax and down-regulation Bcl-2 expression, and activations of poly (ADP-ribose) polymerase (PARP) and caspase cascades. These MeHg-induced apoptotic-related signals could be remarkably reversed by N-acetylcysteine. Western blotting assay showed MeHg increased phosphorylation of ERK1/2 and p38 mitogen-activated protein kinase (MAPK), but not that JNK. Pharmacological inhibitor including: N-acetylcysteine, PD98059, and SB203580 attenuated the MeHg-induced cytotoxicity in neuro-2a cells. Therefore, these results suggests that the signalings of ROS mediated ERK1/2, p38 and mitochondria-dependent apoptosis pathway involve in MeHg-induced neurotoxicity and provide evidence for understanding of MeHg-induced toxicity associated with neurodegenerative diseases.

PS 1394 COMPARATIVE EFFECTS OF METHYLMERCURY AND 1-METHYL-4-PHENYLPYRIDINIUM ON THE DOPAMINERGIC SYSTEM IN MN9D CELLS.

Y. Shao and H. Chan. *Community Health Science Program, University of Northern British Columbia, Prince George, BC, Canada.*

Recent studies have implicated a positive correlation between the prevalence of Parkinson's Disease (PD) and environmental factors such as exposure to pesticides and heavy metals. The objective of this study was to investigate the toxic effects of methylmercury (MeHg) on the dopaminergic system, and to gain a better insight into the mechanism of dopamine regulation linked to PD. The hypothesis is that MeHg would decrease dopamine release in a similar mechanism to that of 1-Methyl-4-Phenylpyridinium (MPP^+), a known agent commonly used to induce pathogenesis of PD, in a dopaminergic neuronal cell line, MN9D. MN9D cells were exposed to MeHg (1–15μM) and MPP^+(10-800μM) for 24h or 48h. Both MeHg and MPP^+ decreased cell viability measured by MTT assay at both time points and dose dependently. Exposure to both MeHg and MPP^+ decreased dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) released by the cells. The DOPAC+HVA/DA ratio also increased with dose. MeHg and MPP^+ down-regulated the expression of DA markers, tyrosine hydroxylase (TH), and dopamine transporter (DAT) at both gene and protein levels. Expression of the α-Synuclein (α-Syn) gene, a hallmark neuropathological indicator of PD, was up-regulated after either treatment. However, protein levels were down-regulated as analyzed by Immunoblot. These findings suggested MeHg can induce pathogenesis of PD similar to that of MPP^+ and the mechanism involves the disruption of DA synthesis, re-uptake, and metabolism processes as well as α-Syn alteration.

PS 1395 CA^{2+} CHANNEL α_1 SUBUNITS DIFFERENTIALLY MODULATE METHYLMERCURY (MEHG)-INDUCED CA^{2+} DYSREGULATION IN HEK-293 CELLS.

H. Hannon, E. M. Sparkenbaugh, K. Krcmarik and W. D. Atchison. *Pharmacology/Toxicology, Michigan State University, East Lansing, MI.*

MeHg causes a biphasic increase of neuronal $[Ca^{2+}]_i$ consisting of Ca^{2+} release from intracellular stores (Phase1- P1) and extracellular Ca^{2+} influx (Phase 2-P2). The $[Ca^{2+}]_i$ increase in P2 is slowed, in part, by nifedipine, ω-conotoxin GVIA, and Ni^{2+}, illustrating the contribution of individual voltage-gated Ca^{2+} channel (VGCC) isoforms in the cellular response to MeHg. Heterologous expression was used in this study to probe discrete effects of low μM concentrations of MeHg on individual VGCC isoforms. HEK 293 cells were transfected with a 1:1:1 M mixture of α_1, $\alpha_2\delta$, and β_3 subunits of human neuronal VGCC cDNA. The α_1 subunit was varied to produce L-, P/Q, and N-type VGCCs ($Ca_v1.2$, 2.1, and 2.2, respectively), corresponding to α_{1C}, α_{1A}, and α_{1B} subunits. Fura-2AM, a ratiometric Ca^{2+} fluorophore, was used to measure $[Ca^{2+}]_i$ in transfected cells exposed to 5 μM MeHg. P1 and P2 time-to-onset was fastest in cells expressing $Ca_v2.2$ (77±30 and 339±71 s, mean ± SEM) compared to those expressing either $Ca_v1.2$ (155±60 and 548±107 s) or $Ca_v2.1$ (155±54 and 452±71 s). Exposure to 2.5 μM MeHg/0 $[Ca^{2+}]_e$ delayed P1 time-to onset in cells expressing $Ca_v2.1$ (278±37 s); the P2 response was eliminated for all isoforms. P1 onset was not affected in either $Ca_v1.2$ or $Ca_v2.2$-expressing cells. The consequences of differential susceptibility of VGCCs to MeHg were also examined using cytotoxicity assays. Transfected cells were exposed to 2 μM MeHg for 3 h and stained in a commercial live/dead assay 24 h later. Cytotoxicity was highest in $Ca_v2.1$-expressing cells (19.0±2.9% dead); $Ca_v2.2$-expressing cells were least affected (6.1±0.4% dead). Together, these results suggest MeHg differentially affects VGCC isoforms to disturb Ca^{2+} homeostasis and induce Ca^{2+}-mediated cell death. Understanding differences in VGCC susceptibility and response will allow a better understanding of selective neuronal vulnerability to MeHg toxicity.

PS 1396 IDENTIFICATION AND CHARACTERIZATION OF MOLECULAR MODULATORS OF METHYLMERCURY-INDUCED DOPAMINE NEURON PATHOLOGY IN *C. ELEGANS.*

N. VanDuyn, R. Settivari, G. Sinclair and R. Nass. *Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.*

Methylmercury (MeHg) exposure from occupational, environmental, and food sources is a significant threat to public health. MeHg poisonings in adults may result in severe psychological and neurological deficits, and in utero exposures can confer embryonic defects and developmental delays. Recent epidemiological and vertebrate studies suggest that MeHg exposure may also contribute to dopamine (DA) neuron vulnerability and the propensity to develop Parkinson's disease (PD). We have developed a novel *Caenorhabditis elegans* (*C. elegans*) model of MeHg toxicity that shows that low, chronic exposure confers embryonic defects, developmental delays, decreases in brood size and animal viability, and DA neuron degeneration. Toxicant exposure results in the robust induction of the glutathione-S-transferases (GSTs) *gst-4*, *gst-5*, *gst-12*, *gst-21*, and *gst-38*, with some GSTs largely dependent on the PD-associated phase II antioxidant transcription factor SKN-1/Nrf2. We also demonstrate that the expression of SKN-1, a protein previously localized to a small subset of chemosensory neurons and intestinal cells in the nematode, is also expressed in the DA neurons, and a reduction in SKN-1 gene expression increases MeHg-induced animal vulnerability and DA neuron degeneration. We will also present our initial studies from 2 genome-wide reverse genetic screens to identify mediators and suppressors of MeHg-induced DA neuron degeneration, as well as our investigations utilizing DA-associated mutants to elucidate the role that the neurotransmitter may play in the toxicity.

PS 1397 ION CHANNEL GENE TRANSCRIPTION IS AFFECTED DIFFERENTIALLY IN RAT CEREBELLUM AFTER POSTNATAL METHYLMERCURY EXPOSURE.

A. Colón-Rodriguez, R. K. Hajela, Y. Yuan and W. D. Atchison. *Pharmacology/Toxicology, Michigan State University, East Lansing, MI.*

Two sets of membrane ion channels are especially sensitive to methylmercury (MeHg): $GABA_A$ receptors and several types of voltage gated Ca^{2+} channels (VGCC), resulting in effects on synaptic transmission and $[Ca^{2+}]i$ homeostasis. We compared the effects of MeHg on gene expression of α1, α6, and δ subunits of the $GABA_A$ receptors, and the pore forming subunits (α1), of the L, N, P/Q and R-type VGCC. Two proteins associated with intracellular Ca^{2+} buffering- Calbindin D28K and the IP3 receptor (IP3R) were also examined. Comparing the expression of the proteins can reveal whether MeHg exposure results in lasting changes in gene expression of nerve membrane proteins for which functional effects of MeHg occur in cerebellum. Rats were treated with 0.75 and 1.5mg/kg/day MeHg sc starting at PND5. Treatment was maintained for 15 or, 30 days, then stopped for a clearing period of 30 days(PND65). Quantitative real time PCR on reverse transcript (cDNA) of RNA isolated from 10 mg of cerebellum was used to assay the transcription of the target protein genes. MeHg did not affect expression of CalbindinD28K or IP3R. All four VGCC subunits were upregulated after 15 days of MeHg, but not at 30 days when compared to the control. Conversely all 3 of the $GABA_A$ receptor subunits showed a downregulation that increased with the treatment and the days of exposure. This appears consistent with our previous observations that MeHg gradually decreases $GABA_A$ receptor-mediated inhibitory postsynaptic potential and inhibitory synaptic transmission to complete block. The upregulation of some VGCC subunits may appear consistent with the fact that MeHg causes increase in $[Ca^{2+}]_i$. The approximately 5-10% increase in the VGCCs and a similar decrease in the $GABA_A$ receptor transcription appeared to be more pronounced in the initial phases of treatment and appeared to stabilize at somewhat less pronounced levels by 60 days. Supported by NIH grants R01ES03299 and R25NS065777.

PS 1398 MOLECULAR MECHANISMS OF IRREVERSIBLE INHIBITION OF SELENOENZYMES BY HIGH METHYLMERCURY EXPOSURES.

N. V. Ralston and L. J. Raymond. *Energy & Environmental Research Center, University of North Dakota, Grand Forks, ND.*

Since the molecular mechanism of methylmercury (MeHg) toxicity has now been identified, it is necessary to refine toxicological understanding of exposure related issues. This study examined the thermodynamic binding affinities and stoichiometries of MeHg and its binding partners in relation to their tissue abundance based on their known physiological abundance in relation to low vs. high MeHg exposures. Methylmercury is a highly specific, irreversible inhibitor of selenium (Se)-dependent enzymes, and its toxic effects appear to be the direct result of interruption of these enzyme activities. The binding affinity between MeHg and the selenium that is the functional component of physiologically essential enzymes is a million times greater than MeHg's affinity for thiomolecules analogues. The high binding affinities and relatively low intracellular abundance of Se make these enzymes extremely vulnerable to high MeHg exposures. The relative binding affinity between MeHg and selenocysteine (Sec) is much greater than for cysteine. (Cys). Because the Se of Sec is the foremost intracellular soft nucleophile, it selectively binds with high affinities to soft electrophiles such as the Hg of MeHg. Instead of being kinetically driven, binding interactions between soft nucleophiles and electrophiles are under thermodynamic control. At equilibrium, the distribution of absorbed MeHg bound with available intracellular Se vs. sulfur is predictable based on thermodynamic affinities and relative intracellular mass abundance. Since ocean fish are among the richest dietary sources of Se, the abundance of Se present in seafoods

needs to be considered when evaluating possible risks associated with MeHg exposures. Since enzyme inhibition is by definition a bimolecular event, risk assessments cannot be performed using the inaccurate assumptions of pseudo-first order reaction rate equations that have previously been applied. In order to be reliable, evaluations of health risks related to MeHg exposures must acknowledge and properly apply the appropriate 2nd order rate equations.

PS 1399 SEPIAPTERIN REDUCTASE AS A TARGET FOR METHYLMERCURY TOXICITY.

S. Yang[1], J. R. Richardson[1], K. Reuhl[2], V. Mishin[2] and J. D. Laskin[1]. *[1]Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ and [2]Pharmacology & Toxicology, Rutgers University, Piscataway, NJ.*

Sepiapterin reductase is an NADPH oxidoreductase important in generating tetrahydrobiopterin, a critical cofactor for enzymes generating neurotransmitters including tyrosine hydroxylase, tryptophan hydroxylase, phenylalanine hydroxylase and the nitric oxide synthases. Mercury toxicity is known to alter levels of neurotransmitters in the brain suggesting that sepiapterin reductase may be an important target for this metal in vivo. We previously demonstrated that mercury chloride is a potent inhibitor of sepiapterin reductase, selectively modifying Cys171 in the putative active center of the enzyme. In the present studies, we compared the inhibitory activity of mercury chloride with methyl mercury, an organic form of the metal. Both mercury chloride and methyl mercury caused a concentration-dependent inhibition of recombinant human sepiapterin reductase (IC50 = 1.8 μM and 4.2 μM, respectively). Glutathione (GSH, 0.1-1 mM) reversed enzyme inhibition. In contrast, selenite, a strong reducing agent reported to antagonize mercury toxicity in animals, had no effect on the sepiapterin reductase inhibition by either mercury chloride or methyl mercury. Both forms of mercury also inhibited partially purified sepiapterin reductase from rat brain and kidney (IC50 = 20 and 25 μM, respectively); with both enzymes, 1 mM glutathione restored ~80% of enzyme inhibition. Sepiapterin reductase was also inhibited in HEK293 cells treated with methyl mercury (IC50 = 35 μM). Buthionine [S,R] sulfoximine (BSO), which reduced levels of GSH >90% in the cells, markedly enhanced enzyme inhibition (IC50 = 12 μM). Methyl mercury was also cytotoxic for HEK293 cells (LC50 = 30 μM); BSO enhanced methyl mercury cytotoxicity (LC50 = 15 μM). Taken together our data further support a role for sepiapterin reductase in mercury toxicity. GSH appears to be important in protecting against mercury toxicity. Supported by CA132624, ES004738, ES005022, GM034310 and AR055073.

PS 1400 LACK OF EFFECT OF ACUTE METHYLMERCURY EXPOSURE ON TYROSINE HYDROXYLASE ACTIVITY IN NIGROSTRIATAL DOPAMINE NEURONS IN MICE.

C. Tiernan[1], M. A. Bernard-Flores[3], J. L. Goudreau[1, 2], K. J. Lookingland[1, 2] and W. D. Atchison[1, 2]. *[1]Neuroscience Program, Michigan State University, East Lansing, MI, [2]Pharmacology & Toxicology, Michigan State University, East Lansing, MI and [3]RISE Program, University of Puerto Rico-Cayey, Cayey.*

Methylmercury (MeHg) causes paresthesia, ataxia, visual dysfunction, and dysarthria. The primary central targets of MeHg are the cerebellum and the visual cortex, however other brain regions are susceptible to its neurotoxic effects. One such area is the nigrostriatal dopamine (NSDA) neuronal system, which arises in the substantia nigra pars compacta (SNc) and terminates in the striatum (ST). Acute exposure to MeHg is reported to alter DA homeostasis in NSDA neurons, increasing the release of DA and decreasing extracellular concentrations of DA metabolites in the striatum. The purpose of the present study was to investigate whether MeHg alters synthesis of DA. To this end, the dose-response and time-course effects of acute MeHg on the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, were examined. Western blot was used to quantify amounts of total TH and its three phosphorylated isoforms, and HPLC-ED was used to determine the concentrations of DA and DOPA in the striatum. In the dose-response study, mice were administered either 4, 8, or 16 mg/kg MeHg by oral gavage 24 h prior to sacrifice. In the time-course study, a single dose of 16 mg/kg MeHg was administered at 6, 12 or 24 h prior to sacrifice. At all doses and times, the amounts of total and phosphorylated TH were unaltered by MeHg administration. Similarly, DOPA accumulation, an in vivo measure of TH activity, was unaffected. These results suggest that MeHg does not alter DA synthesis in NSDA neurons. Interestingly, an increase in DA was observed at 16 mg/kg in the dose-response study, suggesting that MeHg may modulate other steps in DA release and metabolism in NSDA neurons. Supported by funds from the VPRGS at MSU, a ViCTER award to R01ES03299, and R25NS065777.

PS 1401 CHRONIC METHYLMERCURY EXPOSURE OF ADULT MICE DISRUPT HIPPOCAMPAL GLUTAMATERGIC FUNCTIONS.

F. O. Johnson, S. M. Fox and W. D. Atchison. *Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

It is postulated that regular consumption of seafood that is contaminated with the environmental neurotoxicant-methylmercury (MeHg), during adult life could hasten cellular processes associated with brain senescence, age-related neurodegenerative diseases, and deteriorates spatial learning and memory deficits. We investigated whether chronic MeHg exposure during adulthood could hasten adult-onset disruption of [Ca2+]i in hippocampal dentate gyrus and CA3 granule cells leading to aging-related increase in glutamatergic functions. Male mice were exposed daily via drinking water from PND90 to PND270 to 0.0, 0.5 or 5 ppm MeHg. Hippocampal slices were loaded with a Ca2+- selective fluorophore (Fluo-4) and imaged using laser scanning confocal microscopy.
At 5 ppm MeHg but not 0.5 ppm, dentate granule cells exhibit a significant ($p<0.05$) increase in normalized Fluo-4 fluorescence (used as a measure of [Ca2+]i)following puff application of 50 μM NMDA, a glutamate receptor agonist. Whereas normalized Fluo-4 fluorescence of dentate granule cells was significantly larger following treatment with AMPA when compared to NMDA, AMPA caused a nonsignificant fluorescence increase when compared to MeHg treatment groups. Further, treatment with kainate and the glutamate receptor antagonists, CNQX and AP5 had no significant effects on normalized Fluo-4 fluorescence in any MeHg treatment groups. Conversely, CA3 granule cells of MeHg treated groups, showed no significant change in Fluo-4 fluorescence following puff application with any of the glutamate agonists or antagonists. However, NMDA appear to cause a concentration-related increase in Fluo-4 fluorescence signal. These results suggest that chronic MeHg exposure of male mice during adult life can disrupt [Ca2+]i homeostasis by increasing the sensitivity and responsiveness of NMDA receptors in a hippocampal subfield specific manner. Supported by NIH grant R01ES03299.

PS 1402 CHRONIC METHYLMERCURY EXPOSURE DISRUPTS HIGH-RATE RESPONDING ON TWO OPERANT TASKS.

J. Johnson[2] and M. C. Newland[1]. *[1]Auburn University, Auburn University, AL and [2]Duke University Medical Center, Durham, NC.*

Chronic exposure to methylmercury (MeHg) causes sensory-motor deficits that can be detected using operant behavioral techniques. With such preparations, motor and motivational influences over behavior are sometimes conflated. We examine a technique that is thought to distinguish these two influences statistically using a log-survivor analysis of interresponse times. Male BALB/c mice (n=24), housed individually, were 7-8 weeks of age at the start of the experiment and maintained at approximately 85% of their free feeding weight (25-27g). Exposure to 0 or 15 ppm of Hg as methylmercuric chloride dissolved in drinking water began coincident with the start of behavioral testing. Nose-poking was maintained under a multiple schedule arrangement using two approaches to establishing high-rate responding. Under a DRH 9:4, a 9-response burst that occurred within 4 sec produced a conditioned reinforcer and completing 5 of these resulted in sucrose reinforcement. Under a Percentile 10:0.5 schedule, an interresponse time (IRT) that was shorter than half of the previous 10 IRTs was eligible for reinforcement randomly, approximately every 60 sec. The latter approach adjusts the response criterion according to the mouse's recent performance so reinforcers can be earned even if motor function is impaired. The microstructure of behavior was examined using a quantitative model that partitioned responding into bouts characterized by a bout initiation rate (sensitive to motivational influences), within-bout rate (sensitive to motor deficits) and bout length. MeHg lowered within-bout rate after about three months of exposure in both schedules. Effects on bout-initiation rate were less consistent and appeared to have interacted with time-of-day and schedule-type. The partitioning technique is a promising way of separating motor and motivational influences. The percentile schedule is a powerful approach way of maintaining responding even in the face of impairment. (Supported by ES 003299)

PS 1403 GENOTOXICITY AND PRE-NEOPLASTIC LESIONS INDUCED BY 2, 4-DIAMINOTOLUENE IN THE LIVER OF F344 *GPT* DELTA TRANSGENIC RATS.

T. Nohmi[1], N. Toyoda-Hokaiwado[1], T. Inoue[2], K. Masumura[1], H. Hayashi[3], Y. Kawamura[3], Y. Kurata[3], M. Takamune[1], M. Yamada[1], H. Sanada[4], T. Umemura[2] and A. Nishikawa[2]. [1]*Division of Genetics and Mutagenesis, National Institute of Health Sciences, Setagata, Tokyo, Japan,* [2]*Division of Pathology, National Institute of Health Sciences, Setagata, Tokyo, Japan,* [3]*Meiji Seika Kaisha, Ltd., Yokohama, Kanagawa, Japan and* [4]*Safety Research Department, Central Research Laboratories, Kaken Pharmaceutical Co., Ltd., Fujieda, Shizuoka, Japan.*

Transgenic rodent genotoxicity assays are valuable tools to examine genotoxicity of chemicals *in vivo*. Recently, we have established Fischer 344 *gpt* delta rats by backcrossing Sprague Dawley *gpt* delta rats with F344 rats for 15 generations. To begin validation of F344 *gpt* delta rats, we examined the genotoxicity and hepatotoxicity of 2,4-diaminotoluene (2,4-DAT) and 2,6-diaminotoluene (2,6-DAT). Although both compounds are genotoxic in the Ames/Salmonella assay, only 2,4-DAT induces tumors in rat livers. Male F344 *gpt* delta rats were fed diet containing 2,4-DAT at doses of 125, 250, or 500 ppm for 13 weeks, or 2,6-DAT at a dose of 500 ppm for the same period. Mutation frequencies (MFs) of base substitutions, mainly at G:C base pairs, were significantly increased in the livers of 2,4-DAT-treated rats at all three doses. MFs of deletions, mainly one-base deletions, were also increased at 250 and 500 ppm. In contrast, virtually no induction of genotoxicity was identified in the kidneys of 2,4-DAT-treated rats or in the livers of 2,6-DAT-treated rats. Micronucleus assays in the bone marrow were negative with both 2,4-DAT and 2,6-DAT. GST-P-positive foci were detected in the livers of rats treated with 2,4-DAT at a dose of 500 ppm, but not in those treated with 2,6-DAT. These results suggest that genotoxicity should be examined in target organs of rats where carcinogenicity is identified (liver in this case) and also that *gpt* delta transgenic rats may be useful to integrate *in vivo* genotoxicity and pathological toxicity assays, thereby reducing numbers of animals for safety evaluation.

PS 1404 CHEMOPREVENTIVE EFFECTS OF SILYMARIN ON 1, 2-DIMETHYLHYDRAZINE-INDUCED MUTAGENESIS AND CARCINOGENESIS IN THE COLON OF *GPT* DELTA TRANSGENIC RATS.

K. Masumura[1], N. Toyoda-Hokaiwado[1], Y. Yasui[2], M. Muramatsu[1, 3], M. Takamune[1], M. Yamada[1], T. Tanaka[4] and T. Nohmi[1]. [1]*Division of Genetics and Mutagenesis, National Institute of Health Sciences, Tokyo, Japan,* [2]*School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan,* [3]*Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan and* [4]*Tohkai Cytopathology Institute, Gifu, Japan.* Sponsor: A. Nishikawa.

Silymarin, a natural flavonoid from the milk thistle is a prospective chemopreventive agent. We investigated potential chemopreventive effects of silymarin against carcinogenicity and genotoxicity induced by 1,2-dimethylhydrazine (DMH) plus dextran sodium sulfate (DSS) in the colon of F344 *gpt* delta transgenic rats. In the rat model, transgene lambda EG10 DNA carrying the *gpt* gene of *E. coli* is integrated in the chromosome as a reporter for detection of point mutations. Therefore, it is possible to examine anti-genotoxic effects of chemicals in any organs of rats. Seven week-old male F344 *gpt* delta rats were given a single subcutaneous injection of 40 mg/kg DMH, and followed by 1.5% DSS in drinking water for a week. They were fed diets containing 100 or 500 ppm silymarin for 4 weeks, starting one week before DMH injection. At week 4 after the DMH injection, the *gpt* mutant frequencies (MFs) in the colon were increased about 120-fold by DMH/DSS treatments. Silymarin suppressed the induced MFs by 30%, although the reduction was not statistically significant. The number of preneoplastic lesions, i.e., aberrant crypto foci, induced in DMH/DSS groups was significantly decreased by feeding with silymarin. At week 32, DMH/DSS treatments induced colon tumors substantially and silymarin significantly suppressed the tumor formation. *In vitro*, silymarin reduced genotoxicity of DMH by 80% in *S. typhimurium* YG7108, a sensitive strain to alkylating agents. These results suggest that silymarin is able to suppress DMH/DSS-induced colon carcinogenesis when fed at the initiation phase via inhibition of genotoxicity of DMH at least in part, and also that F344 *gpt* delta rats are useful to examine the mechanisms of chemopreventive agents in the target organs of carcinogenesis.

PS 1405 EXAMINING THE MUTAGENICITY OF ETHYL METHANESULFONATE IN MICE USING THE PIG-A, HPRT, AND GPT DELTA ASSAYS.

X. Cao, J. G. Shaddock, R. A. Mittelstaedt, V. N. Dobrovolsky, S. D. Shelton, M. Manjanatha and R. H. Heflich. *DGMT, NCTR/U.S. FDA, Jefferson, AR.* Sponsor: T. Chen.

Ethyl methanesulfonate (EMS) has recently undergone a comprehensive cancer risk assessment that included dose-response measurements of LacZ mutation in Muta™Mouse. This assessment indicated that EMS had a no-effect threshold for genotoxicity of at least 25 mg/kg/day. However, the Muta™Mouse data were characterized by unusually high backgrounds which may have compromised the quantitative risk estimate. Recognizing that assay sensitivity is affected by fold-increases above background, we conducted a series of preliminary in vivo experiments to examine the mutagenicity of EMS in mice using reporter systems with low backgrounds, including Pig-a mutation in RBCs, Hprt mutation in lymphocytes, and transgene mutation induction in gpt delta mice. Male C57BL/6 mice (or gpt delta C57BL/6 transgenic mice) were treated with an acute dose of 360 mg/kg of EMS via oral gavage. Blood samples were obtained on Days -1, 15, 30, and 45 for Pig-a mutation analysis and the spleens were collected on Day 45 for Hprt mutation assay. For the gpt delta assay, the transgenic animals were sacrificed on Day 28 and the bone marrow, spleen, liver, lung, kidney, and small intestine were harvested for mutant analysis. The Hprt assay and spleen gpt assay had the greatest responses with ca. 8-fold increases in mutant frequency (MF) over the background in treated animals. In addition to the spleen, bone marrow also exhibited a statistically significant response to the EMS treatment with a fold induction of 2.3. Results with the Pig-a assay revealed a mutant phenotype erythrocyte response on Day 15 with a fold induction of 6.6. Taken together, these findings suggest that different endpoints provide different levels of sensitivity for detecting the genotoxicity of EMS. Therefore, utilizing a combination of assays with low backgrounds may benefit the dose-response genotoxicity evaluations of weak mutagens such as EMS. Ongoing studies are testing this hypothesis.

PS 1406 MANIFESTATION AND PERSISTENCE OF PIG-A MUTANT FREQUENCY IN C57BL/6 MICE TREATED WITH DIFFERENT DOSES OF ENU.

J. A. Bhalli, M. G. Pearce, V. N. Dobrovolsky and R. H. Heflich. *NCTR, U.S. FDA, Jefferson, AR.* Sponsor: B. Parsons.

Treating rats with single doses of N-ethyl-N-nitrosourea (ENU) results in a time-dependent accumulation of Pig-a-mutant peripheral red blood cells (RBCs), reaching a plateau at about 6-weeks posttreatment and resulting in a nearly linear dose-response, with the magnitude of the induced response persisting for at least 26 weeks. In the present study, we have conducted a detailed evaluation of ENU-induced Pig-a mutant manifestation and persistence in the mouse. Groups of 5 male C57BL/6 mice were given single i.p. injections of seven different doses of ENU: 0 (vehicle control), 10, 25, 45, 70, 100 and 140 mg/kg ENU. Blood samples were collected one day prior to treatment and at 2, 4, 6, 8, 12, 20 and 26 weeks posttreatment and analyzed for CD24Neg RBCs and CD24Neg reticulocytes (RETs) (Pig-a mutants) using a FACSCanto-II flow cytometer. Mean CD24Neg frequencies in vehicle-treated mice ranged from $0\times10-6$ to $1.8\times10-6$ for RBCs and $0\times10-6$ to $4\times10-6$ for RETs, and displayed no time-related trends. For RETs, maximum Pig-a mutant frequencies were observed at the 2nd week after ENU treatment, whereas maximum Pig-a mutant frequencies for RBCs were observed at the 4th week after treatment; mutant (CD24Neg) RET frequencies were consistently approx. twice those of RBC frequencies throughout the study period. After the maximum responses were reached, the CD24Neg RBC and RET frequencies in ENU-treated mice began to slowly decrease with time. Although significant, dose-dependent increases in Pig-a mutant frequency were observed for all doses of ENU, the dose-response appeared to be sublinear at lower doses. In addition, the absolute magnitude of the ENU-induced Pig-a mutant RBC response in mice was about 2-3-fold less than previously detected in rats. The data from this study indicate that the ENU-induced Pig-a mutant RBC frequency in mice and rats differ in magnitude, manifestation kinetics, and persistence.

PS 1407 NITROXIDES TEMPO AND TEMPOL INDUCE TK MUTATIONS IN MOUSE LYMPHOMA CELLS.

X. Guo[1], L. Guo[2], M. M. Moore[1] and N. Mei[1]. [1]*Division of Genetic and Molecular Toxicology, NCTR/FDA, Jefferson, AR and* [2]*Division of Biochemical Toxicology, NCTR/FDA, Jefferson, AR.*

Low molecular weight nitroxides, Tempo and Tempol (4-OH-Tempo), are stable free radical compounds widely used throughout chemistry and biochemistry as process intermediates. Due to their antioxidant abilities and protective properties in

various pathological situations, some studies have proposed them as potential therapeutic agents in medical applications. Thus, the potential risks of uses of these nitroxides need to be addressed. In this study, we investigated the cytotoxicity and mutagenicity of Tempo and Tempol using the mouse lymphoma assay. In the absence of metabolic activation, the L5178Y/Tk+/—3.7.2C mouse lymphoma cells treated with 3 mM Tempo showed significant cytotoxicity and marginal mutagenicity. In the presence of S9, the treatment of cells with Tempo at concentrations of 1-2 mM resulted in a decrease of the relative total growth (RTG) and an increase of mutant frequency. Treatment of cells with Tempol at concentrations of 2-10 mM with or without S9 resulted in dose-related increases of both cytotoxicity and mutagenicity. Within the dose range tested, oxidative stress induced by Tempo and Tempol was detected in the mouse lymphoma cells. Both compounds showed significantly reduced glutathione (GSH) levels in a dose-related manner in the all treatment groups. In addition, Tempo resulted in approximately a 1.5-fold increase of the reactive oxygen species (ROS) in all treatment doses. To explore the underlying mutagenic mechanism, we examined the loss of heterozygosity (LOH) at four microsatellite loci spanning the entire chromosome 11 for the Tk mutants induced by Tempo and Tempol. The mutational spectra induced by both compounds, in which the majority of mutants showed LOH at the Tk locus only (i.e., chromosome damage less than 30 centimorgan), were distinctly different from the vehicle control. These results suggest that Tempo and Tempol are mutagenic in mouse lymphoma cells with a clastogenic mode-of-action.

PS 1408 GENOTOXICITY OF 3, 5-DIMETHYLANILINE AND ITS METABOLITES.

M. Chao, L. J. Trudel and G. N. Wogan. *Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA.*

Epidemiological studies have demonstrated extensive human exposure to the monocyclic aromatic amine, 3,5-dimethylaniline (3,5-DMA). Although some aromatic amines such as 4-aminobiphenyl are well-documented risk factors for bladder cancer, little is known about the genotoxicity and potential carcinogenic risk created by 3,5-DMA exposure. We therefore are investigating the cytotoxicity and mutagenicity of 3,5-DMA and its metabolites N-hydroxyl-3,5-dimethylaniline (N-OH-3,5-DMA) and 3,5-dimethylaminophenol (3,5-DMAP) in Chinese hamster ovary (CHO) cells. Cytotoxicity and aprt mutagenicity induced by these chemicals in nucleotide repair-proficient (RP) AA8 and repair-deficient (RD) UV5 cell lines. These cells do not express significant levels of phase I and II enzymes necessary for xenobiotic activation. Results to date show that the parental chemical, 3,5-DMA, as well as the N-OH-3,5-DMA and 3,5-DMAP derivatives all caused dose-dependent decreases in cell survival, but with very different potencies: 3,5-DMAP had the highest potency; N-OH-DMA intermediate; and 3,5-DMA lowest. No aprt mutations were induced by these treatments, even at highly toxic doses. It was noted however, that exposure to these compounds induced dose-dependent production of ROS, as determined by carboxyl-H2DCF-DA fluorescence. To assess the contributions of phase I and II enzymes to the toxicity of these alkylanilines, experiments as described above were conducted in isogenic AA8 and UV5 cells genetically modified to express human cytochrome P4501A2 (CYP1A2) and N-acetyltransferase-2 (NAT2). Treatment with the alkylanilines again caused dose-dependent cell killing, with 3,5-DMAP being most potent, N-hydroxy-3,5-DMA intermediate and 3,5-DMA least potent. All three compounds also induced substantial increases in aprt mutation frequency at dose levels reflecting their cytotoxic potencies. In summary, these results indicate that 3,5-DMA and its metabolites are cytotoxic to the cells and require CYP1A2 and NAT2 to exert their mutagenic effects.

PS 1409 COMPARISON OF THE BACTERIAL MUTAGENICITY OF MAINSTREAM WHOLE SMOKE FROM CIGARETTES WITH DIFFERENT LEVELS OF MENTHOL.

R. Leverette. *Lorillard, Greensboro, NC.*

Menthol is widely used in the pharmaceutical, cosmetic, food and tobacco industries and is generally regarded as safe (GRAS) for these applications. This study was conducted to compare the mutagenicity of mainstream whole-smoke (WS) and wet total particulate matter (WTPM) from two sets of experimental cigarettes to determine if menthol has an effect on this endpoint. The first pair of experimental cigarettes (delivering ~6mg WTPM/cig) included a mentholated (0.6% w/w menthol) and a comparable non-mentholated control. The second set consisted of four experimental cigarettes (delivering ~9mg WTPM/cig) with increasing levels of menthol (0.1% - 0.7% w/w) and a non-mentholated control. Within each set, the cigarettes were comparable in construction, composition, and WTPM deliveries with added menthol spanning typical user levels. All cigarettes were smoked under ISO puff profile (35mL volume, 2 second duration and 1 minute interval) on a VITROCELL® VC10 smoking robot, with WTPM being pad collected and extracted in dimethylsulfoxide. TA98 and TA100 were exposed to WS or WTPM with metabolic activation (S9+) utilizing either the VITROCELL® Ames exposure modules and whole-smoke (WS) dilution system or the preincubation method (WTPM). The WS specific activity (revertants/ug) from the first pair of sample cigarettes showed the mentholated cigarette had significantly lower activity then the non-mentholated control (TA98, $p = 0.0027$; TA100, $p = 0.0297$). For the second set of cigarettes, only small differences in WS activities were observed, with a trend of decreasing activity with increasing menthol that was not statistically significant. No differences were detected for WTPM specific activities. No significant differences in WS activities occurred when cigarettes were removed from packs and conditioned (18 hours, 23°C, 60% RH) prior to smoking. With no changes in smoke delivery or cytotoxicity and with the reduction of menthol from the tobacco during conditioning, these results suggest menthol may have a role in the observed reduction of WS Ames activity.

PS 1410 A NOVEL *IN VITRO* SYSTEM FOR THE TOXICOLOGICAL EVALUATION OF GENOTOXIC COMPOUNDS.

S. O. Mueller, K. Boehme, Y. Dietz and P. G. Hewitt. *Toxicology, Merck KGaA, Darmstadt, Germany.*

Genotoxicity is an important issue during pharmaceutical development and chemical risk assessment. In vivo carcinogenicity studies are animal, time and cost-intensive and in vitro tests have relatively low specificity. Furthermore, economic and animal welfare aspects, in particular within the scope of REACH, indicate the importance of new in vitro tools. Previous studies have proven the suitability of toxicogenomic approaches for the predictive classification of genotoxicants in vivo, but in vitro prediction is still a challenge.

This study aimed to develop a new genomics-based test system for mutagens and promutagens in vitro using HepG2 cells. Due to their limited metabolic capacity we established a combined system of HepG2 cells and a metabolic activation system (MAS – rat liver S9) for promutagen testing. P53 activation in nuclear protein extracts of HepG2 cells served as surrogate marker for compound-related genotoxicity and has confirmed the functionality of the MAS-HepG2 system. Global gene expression changes were quantified 24h and 48h after treatment with (pro-)genotoxic (cyclophosphamide, dimethylbenz[a]¬anthracene, Aflatoxin B1, actinomycin D, methyl methanesulfonate, etoposide) and non-genotoxic (metformin, theophylline) compounds using Illumina BeadChip arrays. Differential gene regulations between both compound classes were identified by gene Ranking with an ANOVA for group separation followed by support vector machine algorithm for classifier calculation. The best classification was achieved using the 91 top-scored genes. The predicitvity was evaluated by k-fold cross validation, showing an appropriate group allocation according to the specified annotation. Thereafter, the classifier built was used to predict the toxicity of diethylnitrosamine correctly.

The 91 genes identified are potential candidates for the mechanistic characterization and identification of new genotoxicants. Combined with other genotoxicity endpoints as well as the inclusion of metabolic activation might enable a holistic screening system for a broad range of potentially genotoxic compounds.

PS 1411 COMPARATIVE MUTAGENICITY OF CYCLOPHOSPHAMIDE IN F344 MALE RATS USING THE PIG-A AND HPRT ASSAYS.

V. N. Dobrovolsky, J. G. Shaddock, M. Pearce and R. H. Heflich. *NCTR, Jefferson, AR.* Sponsor: B. Parsons.

Cyclophosphamide (CP) is a bifunctional alkylating agent that forms interstrand crosslinks in double-stranded DNA. Even though it is a potent clastogen and known human carcinogen, CP is frequently used for cancer chemotherapy and to a lesser degree for treatment of autoimmune diseases. In the present study, we administered single i.p. doses of 10, 40 and 80 mg/kg CP to male F344 rats and evaluated its ability to induce gene mutation in two assays that employ X-linked endogenous reporter genes, the red blood cell (RBC) Pig-a assay and the lymphocyte Hprt assay; the reticulocyte (RET) micronucleus assay for clastogenicity/aneugenicity was performed for comparison. Micronucleated (MN) RETs and percent RETs (as a measure of cytotoxicity) were assayed by flow cytometry in peripheral blood 48 hr posttreatment, while the Pig-a assay (measuring the frequency of CD59-deficient peripheral RBCs) was conducted using flow cytometry at 1, 2, 3, 4, 6, 8, 9, 12 and 16 weeks posttreatment, and the frequency of 6-thioguanine-resistant (Hprt mutant) spleen T-cells was determined in clonal assays at 3, 6, 9 and 16 weeks. CP treatment produced a strong short-term suppression of erythropoiesis and 10-fold increase in MN RET frequency (0.07% in untreated controls to ca. 0.8% in treated animals). The RBC Pig-a mutant frequency was marginally ele-

vated only in high-dose-treated animals (max. 7.5×10-6 vs 2.7×10-6 control) and only when measured at 6, 8 and 9 weeks post-treatment. The Hprt mutant frequency was elevated in all high-dose-treated animals at all measured time-points (max, 41×10-6 vs 4.7×10-6 control) and in mid-dose-treated animals at 16 weeks (the only time measured). The results suggest that the two gene mutation assays differ in their ability to detect the genotoxicity of CP. Potential explanations for such a differential response will be discussed.

PS 1412 INTERNATIONAL, INTERLABORATORY *PIG-A* MUTATION ASSAY TRIAL: EVALUATION OF TRANSFERABILITY.

S. Dertinger[1], S. Phonethepswath[1], P. Weller[1], L. Stankowski[2], D. Roberts[2], J. Shi[3], L. Krsmanovic[3], H. Vohr[4], L. Custer[5], C. Gleason[5], A. Henwood[5], K. Sweder[5], A. Giddings[6], A. Lynch[6], W. Gunther[7], C. Thiffeault[7], T. Shutsky[7], R. Fiedler[7], J. Bhalli[8], R. Heflich[8], J. Nicolette[9], P. Sonders[9], J. Murray[9], T. Kimoto[10] and J. MacGregor[11]. *[1]Litron Laboratories, Rochester, NY, [2]Covance Laboratories, Vienna, VA, [3]BioReliance, Rockville, MD, [4]Bayer Schering Pharmacology AG, Wuppertal, Germany, [5]Bristol-Myers Squibb, Syracuse, NY, [6]GlaxoSmithKline, Ware, United Kingdom, [7]Pfizer Global R&D, Groton, CT, [8]U.S. FDA-NCTR, Jefferson, AR, [9]Abbott Laboratories, Abbott Park, IL, [10]Teijin Pharmacology Tokyo, Japan and [11]Toxicology Consulting Services, Arnold, MD.*

An *in vivo* mutation assay has been developed based on the enumeration of CD59-negative erythrocytes (indicative of *Pig-a* gene mutation). In this system, anti-CD59-PE and SYTO 13 dye are used to label leukocyte-depleted blood samples, and the frequency of CD59-Neg erythrocytes (RBCs) and reticulocytes (RETs) are determined *via* flow cytometry. To evaluate transferability, ten labs determined the effect of N-ethyl-N-nitrosourea (ENU) administered to male rats *via* oral gavage for 3 consecutive days. All labs studied 0, 20 and 40 mg/kg/day (n = 5/group), and two also studied ENU at 4 mg/kg/day. Serial blood samples were collected on Days -1, 15, & 30, with some labs also sampling on Days 45 & 90. Samples were processed according to standardized methods and data acquisition protocols, and 3 endpoints were measured: %RETs and CD59-Neg RET & RBC frequencies. Each lab observed dose-related increases in the frequency of CD59-Neg RETs and CD59-Neg RBCs on Day 15. Maximal CD59-Neg RET responses tended to occur by Day 15, whereas peak CD59-Neg RBC responses were achieved by approximately Day 45. Elevated CD59-Neg RET and RBC frequencies were maintained through the latest time-point studied (Day 90). These data demonstrate close correspondence among labs, both in terms of the timing and magnitude of the responses, and illustrate that this assay is robust and readily transferable across sites. Additional interlab studies are in progress that will increase the number of chemicals evaluated in order to systematically characterize assay performance.

PS 1413 TOXICOLOGICAL CHARACTERIZATION OF DIESEL ENGINE EMISSIONS USING BIODIESEL AND A CLOSED SOOT FILTER.

I. M. Kooter, M. A. van Vugt, A. D. Jedynska, R. P. Verbeek and C. A. Krul. *TNO, Utrecht, Netherlands.* Sponsor: R. Woutersen.

This study was designed to determine the toxicity (oxidative stress, cytotoxicity, genotoxicity) in extracts of combustion aerosols. A typical Euro III heavy truck engine was tested over the European Transient Cycle with three different fuels: conventional diesel EN590, biodiesel EN14214 as B100 and blends with conventional diesel (B5, B10, and B20) and pure plant oil DIN51605 (PPO). In addition application of a (wall flow) diesel particulate filter (DPF) with conventional diesel EN590 was tested. The use of B100 or PPO as a fuel or the DPF reduced PM mass and numbers over 80%. Similarly, significant reduction in the emission of chemical constituents (EC 90%, (oxy)-PAH 70%) were achieved. No significant changes in nitro-PAH were observed. The use of B100 or PPO led to a NOx increase of about 30%, but no increase is found for DPF application. The effects of B100, PPO and the DPF on the biological test results vary strongly from positive to negative depending on the biological end point. The oxidative potential, measured via the DTT assay, of the B100 and PPO or DPF emissions is reduced by 95%. The cytotoxicity is increased for B100 by 200%. The measured mutagenicity, using the Ames assay test with TA98 and YG1024 strains of Salmonella typhimurium indicate a dose response for the nitroarene sensitive YG1024 strain for B100 and PPO (fold induction: 1.6). In summary B100 and PPO have good potential for the use as a second generation biofuel resulting in lower mass exhaust gas, similar to application of DPF, but caution should be made due to potential increased toxicity. Besides regulation via mass, the biological reactivity of exhaust emissions of new (bio)fuels and application of new technologies, needs attention. The different responses of different biological tests as well as differences in results between test laboratories underline the need for harmonization of test methods and international cooperation.

PS 1414 *IN VITRO* PREDICTIONS OF *IN VIVO* GENOTOXICITY ARE BETTER THAN PREDICTIONS OF CARCINOGENICITY.

R. Walmsley[1, 2]. *[1]Life Sciences, University of Manchester, Manchester, United Kingdom and [2]Gentronix Ltd., Manchester, United Kingdom.*

The sensitivity and specificity of regulatory in vitro assays in the prediction of carcinogenicity were calculated by Kirkland et al (2005), using the carcinogenicity potency database (CPDB). in vitro mammalian tests had higher sensitivities (70-90%) than Ames (60%), but produced positive results for more non-carcinogens (~50%) than Ames (77%). Hence Ames positive pharmaceutical candidates rarely proceed to animal testing, whereas Ames negative in vitro mammalian test positives often proceed. Genotoxicity assays are not expected to predict carcinogenicity where tumours arise by a non-genotoxic mode of action. However, in vivo genotoxicity prediction avoids the need for this distinction, and its accuracy is equally important in making decisions regarding the fate of candidate pharmaceuticals. This study presents in vivo sensitivity and specificity figures for 4 commonly used in vitro assays in a collection of 155 compounds.

Ames predictions for in vivo genotoxicity and carcinogenicity (Kirkland's in brackets) were similar in sensitivity, 58% (59-60%), though specificity was higher, 86% (74-77%). The GADD45a-GFP 'GreenScreen HC' assay predictions were similar for sensitivity, 85% (88% Hastwell, 2009) and specificity, 92% (95%, Hastwell 2009). MLA showed slightly better in vivo sensitivity, 86% (73– 81%), and considerably increased specificity, 79% (39–48%). MNT/CA showed similar improvements in sensitivity, 90% (79– 81%), though specificity remains low 65% (31–55%).

The generally higher sensitivity to in vivo genotoxins (cf carcinogens) probably reflects the expectation that in vitro assays will produce negative results for non-genotoxic carcinogens. The higher in vivo specificity figures might reflect the greater prevalence of pharmaceuticals in this collection (cf CPDB), which are generally less reactive. The GADD45a-GFP assay has the highest specificity, and hence the most reliable positive results. It is already used in screening, but might usefully be applied to compounds uniquely positive in MLA/MNT/CA, to identify those most likely to be in vivo positive.

PS 1415 CYTOTOXICITY AND GENE EXPRESSION ALTERATIONS FOLLOWING TREATMENT OF TK6 CELLS WITH ETOPOSIDE AND SODIUM CHLORIDE.

Y. Chen[1], M. M. Moore[1] and J. C. Fuscoe[2]. *[1]Division of Genetic and Molecular Toxicology, U.S. FDA/NCTR, Jefferson, AR and [2]Division of Systems Biology, U.S. FDA/NCTR, Jefferson, AR.*

The use of gene expression data in determining the relevance of genotoxic damage is gaining momentum. The dose at which gene expression is evaluated is a key question. Using TK6 cells and 4 genes involved in genotoxic stress response (p21, GADD45, ATF3, and DDB2) we are investigating whether gene expression alterations can be observed at chemical concentrations inducing levels of cytotoxicity that would be considered acceptable (biologically relevant) in the standard gene mutation assays. Cells were treated with etoposide (0.1, 0.4, 1.1, 3.3 and 10 ug/ml) and sodium chloride (0.1, 0.4, 1.1, 3.3 and 10 mg/ml). Both MTS and plating efficiency were used to assess cytotoxicity. Based on MTS, the top concentrations of the two chemicals induced approximately 50% cytotoxicity. Plating efficiency assessment revealed that the top two concentration of etoposide resulted in greater than 95% cytotoxicity and the top concentration of sodium chloride, substantially higher than the recommended concentration for in vitro mammalian cell assays, resulted in greater than 90% cytotoxicity. For etoposide treated cells there were small increases in the expression of all 4 genes at the first 3 concentrations with dramatic increases seen only at concentrations causing very high cytotoxicity. For sodium chloride, significant up- regulation of the 4 genes only occurred at the 10 mg/ml concentration. Etoposide is an extremely potent clastogen while sodium chloride only induces genetic damage at high concentrations causing alterations in osmolality. Given the small magnitude of gene expression changes seen with etoposide, it is important that a large number of additional chemicals be assessed before conclusions can be drawn concerning the utility of this gene expression approach to detecting genotoxicants.

PS 1416 PIG-A MUTATION AND MICRONUCLEATED RETICULOCYTE ASSAYS DISCRIMINATE THE MUTAGENIC/NON-MUTAGENIC PAIR: BENZO[A]PYRENE/PYRENE.

D. Torous[1], J. MacGregor[2], S. Phonethepswath[1], P. Weller[1], J. Bemis[1] and S. Dertinger[1]. [1]*Litron Laboratories, Rochester, NY and* [2]*Toxicology Consulting Services, Arnold, MD.*

Integration of genotoxicity endpoints into general toxicology studies is attractive for several reasons, including the potential to reduce animal use and provide comprehensive toxicity information that aids interpretation of genotoxicity results. This laboratory has developed automated scoring techniques for monitoring two cross-species, blood-based genotoxicity endpoints, thereby making integration practical: flow cytometric procedures for scoring micronucleated reticulocyte frequency and gene mutation at the Pig-a locus. The ability to integrate these endpoints into a 28-day repeat dosing schedule was investigated with a mutagenic/non-mutagenic pair at maximum tolerated dose levels: benzo[a]pyrene (BP, 150 mg/kg/day) and pyrene (Pyr, 125, 250 and 500 mg/kg/day). Male Wistar Han rats were treated on Days 1-28 via oral gavage. Blood samples obtained on Days -1, 15, 29 and 56 were analyzed for Pig-a mutation with a dual staining method (SYTO 13 in combination with anti-CD59-PE) that facilitated mutant cell frequency measurements in both total erythrocytes and the reticulocyte subpopulation. Day 4 and 29 specimens were evaluated for micronucleus frequency according to MicroFlow® Kit instructions. BP induced robust responses on Days 15, 29 and 56 in the mutant phenotype reticulocyte population, and on Days 29 and 56 in the mutant phenotype erythrocyte population. No mutagenic effects in either population were apparent for Pyr. Significant increases in micronucleated reticulocyte frequencies were observed with BP on Days 4 and 29, whereas Pyr had no effect. These results suggest that both endpoints are relatively specific for genotoxic activity, as each discriminated the structurally-related mutagenic and non-mutagenic agents, even when treatment was at a maximum tolerated dose.

PS 1417 THE MUTAGENIC POTENTIAL OF CIS-2-BUTENE-1, 4-DIAL.

A. Terrell[1], M. Huynh[2] and L. A. Peterson[1, 3]. [1]*Environmental Health Sciences, University of Minnesota, Minneapolis, MN,* [2]*Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN and* [3]*Masonic Cancer Center, University of Minnesota, Minneapolis, MN.*

Furan is a known rodent hepatotoxicant and carcinogen. Human exposure to furan is widespread, but the human health effects are unknown. While there have been many studies of furan toxicity, the mechanism of carcinogenicity is still debated. Both genotoxic and nongenotoxic mechanisms have been proposed but in vivo and in vitro studies have reported conflicting and inconclusive results. It is suspected that the reactive metabolite of furan, cis-2-butene-1,4-dial, is the toxic and carcinogenic agent. In this study, the Big Blue in vitro mutagenesis model was used to evaluate the mutagenic potential of a single dose and chronic dose of cis-2-butene-1,4-dial. Overall, the results suggest that cis-2-butene-1,4-dial is not genotoxic in the Big Blue in vitro mutagenesis model at the tested treatment. [funded by ES10577]

PS 1418 THE METABOLITES OF THE AZO DYE DISPERSE RED 1 CAN REPRESENT HUMAN RISKS CONSIDERING THE INGESTION OF CONTAMINATED WATER AND FOOD.

F. D. Chequer[1], T. M. Lizier[2], M. B. Zanoni[2], R. Marcos[3] and D. P. Oliveira[1]. [1]*Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil,* [2]*(2) Universidade Paulista Júlio de Mesquita Filho, Araraquara, São Paulo, Brazil and* [3]*(3) Universitat Autònoma de Barcelona, Barcelona, Spain.*

Introduction: Azo dyes constitute the largest class of synthetic dyes used in commercial applications. Following oral exposure, these dyes can be reduced to aromatic amines by the intestinal microflora or liver azoreductases. Objectives: To determine the products formed after oxidation and reduction of the dye Disperse Red 1, simulating hepatic biotransformation; and to evaluate the mutagenic potential of the resultant solution. Methods: Controlled potential electrolysis was carried out on dye solution using a Potentiostat/Galvanostat. HPLC-DAD was used for the determination of aromatic amines generated after oxidation/reduction process. The Salmonella/microssome assay with the strain TA98 and YG1041 with and without S9, as well as mouse lymphoma assay (MLA) using the thymidine kinase (Tk) gene were used for the evaluation of the mutagenicity of the solution obtained after the reduction/oxidation process. Results: We have identified Sulfate de N-ethyl-(2-hidroxiethyl)-p-phenyldiamine monohydrate and Nitrobenzene resultants from oxidation and reduction of the dye Disperse Red 1. Our group showed that this azo dye is mutagenic in different cell system. In addition after the oxidation/reduction process, the dye still has mutagenic activity for the Salmonella/microssome assay. We observed that the mutagenic potential of the resultant solution to the strain YG1041 decreased after both the processes compared with the original dye, while for the strain TA98 we observed an increase of the effect. Yet, both the original dye Disperse Red 1 and its treated solutions showed negative results in the MLA. Conclusions: Considering that the reduction/oxidation processes are similar to hepatic reaction, we concluded that the ingestion of water and food contaminated with this dye is a human health problem.
Financial support:CNPq

PS 1419 MUTATION SPECTRUM IN HPRT-/GPT+ V79 CELLS TREATED WITH TEAK WOOD EXTRACT AND 2-METHYLANTHRAQUINONE, AN ABUNDANT BIQUINONE IN TEAK WOOD.

M. J. Wilson[1], C. Miller[1, 2], G. Sabbioni[1, 2] and R. Rando[1]. [1]*Environmental Health Sciences, Tulane University, New Orleans, LA and* [2]*Tulane Cancer Center, Tulane University, New Orleans, LA.*

Wood dusts are generally classified as known human carcinogens by NTP and IARC based on a strong association with increased risk of nasal cancer. However, no specific carcinogenic compounds have been identified in wood dusts. Teak wood (Tectona grandis) is an important tropical hardwood frequently used in furniture manufacturing. An increase in mutation frequency was observed in a hprt-/gpt+ cell line following treatment with methanol extract of teak wood dust. Fractionation of the teak dust extract using multiple analytical methods identified 2-methylanthraquinone as an abundant bioactive constituent. Large deletions of the gpt transgene were caused by teak wood dust extract or 2-methylanthraquinone treatments. These large signature deletions were three-fold higher in the 2-methylanthraquinone and teak extract treatments. We conclude that teak wood dust and its major toxic constituent, 2-methylanthraquinone, are mutagenic and may share a common mechanism of action. 2-methylanthraquinone has not been formally identified as a mutagen or carcinogen, but anthraquinone and other substituted derivatives possess these activities. The presence of an abundant anthraquinone implies teak dusts may pose an exceptional occupational risk to woodworkers.

PS 1420 GENOTOXICITY ASSESSMENT OF ETHYLENEDIAMINE DINITRATE (EDDN) AND DIETHYLENETRIAMINE TRINITRATE (DETN).

G. Reddy[1], J. Song[2], P. Kirby[2] and M. S. Johnson[1]. [1]*Directorate of Toxicology, U.S. Army Public Health Command, Aberdeen Proving Ground, MD and* [2]*SITEK Research Laboratories, Rockville, MD.*

Ethylenediamine dinitrate (EDDN) and Diethylene triamine trinitrate (DETN) are explosive compounds used in combination with other high explosives as safe, insensitive, and inexpensive replacements. The genetic toxicity of these compounds was evaluated to predict the potential for adverse human health effects from exposure. The genetic toxicity test battery included: gene mutation test in bacteria (Ames), in vitro chromosome aberration test and in vivo mouse micronucleus test. The results of the Ames test indicated DETN was negative for Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537, and Escherichia coli strain WP2 uvrA at doses up to 5000 µg/plate both with and without metabolic activation. EDDN produced a positive increase in the mean number of revertants per plate with the tester strain TA100 without activation (3.1-fold) at 5000 µg/plate. No positive increases were observed with the other tester strains with or without activation. The Chinese Hamster Ovary Cell (CHO) Chromosome Aberration Assay was performed using DETN at concentrations up to 5000 µg/mL. The results indicated that DETN did not induce structural chromosomal aberrations at concentrations up to 5000 µg /mL in CHO cells, with or without metabolic activation. EDDN tested up to 5000 µg/mL both without and with metabolic activation, produced negative results in CHO cells. DETN and EDDN, when tested in vivo in the CD-1 mouse at doses up to 2000 mg/kg, did not induce a significant increase in the number of micronuclei in bone marrow erythrocytes. These studies demonstrate that EDDN is mutagenic in one specific strain of Salmonella (TA100) but were negative in other strains and in vitro for induction of chromosomal aberrations in CHO cells and for micronuclei in the in vivo mouse micronucleus assay. DETN was not genotoxic in both in vitro and in vivo tests. These results suggest a low risk of genetic hazards associated with exposure to these chemicals.

PS 1421 DOSE-RESPONSE OF NAPHTHALENE–INDUCED GENOTOXICITY AND GLUTATHIONE DETOXIFICATION IN HUMAN TK6 LYMPHOBLASTS.

L. Recio[1], K. Shepard[1], C. Swartz[1] and G. Kedderis[2]. [1]*ILS, Research Triangle Park, NC and* [2]*Consultant, Chapel Hill, NC.*

Naphthalene is bioactivated by CYP450s in vivo and in vitro to a spectrum of cytotoxic and genotoxic metabolites. We are determining the dose-response relationship for naphthalene-induced genotoxicity by assessing the induction of micronuclei (MN) using a multiplex cytotoxicity and genotoxicity flow cytometry based assay. This assay quantifies percent apoptosis/necrosis, cell cycle, relative percent survival and MN in the same sample. For these studies, TK6 cells (2 x 105 cells/mL in 3 mL of RPMI 1640 with B-naphthoflavone/phenobarbital (BNP/PB)-induced rat liver S9 and a NADPH-generating system) in glass 25 mL Erlenmeyer flasks, were exposed to a range of naphthalene concentrations (0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, 30.0 uM) in triplicate using methanol as the vehicle. A significant non-linear increase in naphthalene-induced MN was observed with no significant increases in MN at less than 20 uM and significant dose dependent increases in MN at 20 and 30 uM naphthalene with 50-60% relative cell survival. Supplementation of the exposure system with 5 mM glutathione effectively eliminated naphthalene cytotoxicity and genotoxicity at these exposure levels shifting the dose-response greater than 10-fold; significant cytotoxicity and genotoxicity was observed at 500 uM compared to 30 uM in the absence of glutathione. These data demonstrate that naphthalene bioactivated by the commonly used BNP/PB-induced rat liver S9 exhibits a nonlinear dose-response for the induction of MN with a point of departure from the background frequency of MN in human TK6 cells at ≥ 20 uM. The genotoxicity of naphthalene is effectively eliminated at these exposure levels in the presence of glutathione, demonstrating the key role of glutathione in the detoxication of naphthalene cytotoxicity and genotoxicity. Supported by the Naphthalene Research Council)

PS 1422 DIMETHYLSULFOXIDE (DMSO) INHIBITION OF NAPHTHALENE-INDUCED CYTOTOXICITY AND GENOTOXICITY IN HUMAN TK6 CELLS.

K. Shepard[1], G. Kedderis[2], C. Swartz[1] and L. Recio[1]. [1]*ILS, Research Triangle Park, NC and* [2]*Consultant, Chapel Hill, NC.*

Naphthalene is bioactivated by CYP450s in vivo and in vitro to a spectrum of cytotoxic and genotoxic metabolites. We are determining the dose-response relationship for naphthalene-induced genotoxicity by assessing the induction of micronuclei (MN) using a multiplex cytotoxicity and genotoxicity flow cytometry based assay. This assay quantifies percent apoptosis/necrosis, cell cycle, relative percent survival and MN in the same sample. For these studies, TK6 cells (2 x 105 cells/mL in 3 mL of RPMI 1640 with B-naphthoflavone/phenobarbital-induced rat liver S9 and a NADPH-generating system), were exposed to a range of naphthalene concentrations in glass 25 mL Erlenmeyer flasks. Initial studies used DMSO (1% final concentration) as the solvent for assessing naphthalene (100 uM - 1.0 mM) genotoxicity; 750 uM, naphthalene was cytotoxic and showed equivocal genotoxicity. However, when methanol (1% final concentration) was used as the solvent, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 30 uM naphthalene was tested and a significant non-linear increase in MN was observed at 20 and 30 uM with 50% relative survival. Since DMSO is a recommended and common solvent for in vitro toxicology testing and high-throughput test systems (HTS), we further examined the impact of DMSO as the solvent for naphthalene at 0.5% and 0.25% final concentration compared to methanol at 1%. With DMSO (0.5 and 0.25%) as the naphthalene solvent, 400 uM naphthalene gave a similarly cytotoxic and genotoxic response as that observed with 30 uM naphthalene when methanol (1%) was used as the solvent. These results demonstrate the inhibition of naphthalene cytotoxicity and genotoxicity when using DMSO as the solvent compared to methanol, shifting the dose-response for cytotoxicity and genotoxicity greater than 10-fold higher. These data suggest that the use of DMSO as the universal solvent for in vitro toxicology testing and metabolically competent HTS can quantitatively impact the observed biological response. (Supported by the Naphthalene Research Council)

PS 1423 *IN VIVO* MRI OF RAT THYROID GLANDS AND CORRELATION WITH HISTOPATHOLOGY.

M. Stephan-Gueldner, B. Kuenekke, B. Lenz, A. Braendli-Baiocco, J. Funk, M. vonKienlin and T. Pfister. *F. Hoffmann-La Roche AG, Basel, Switzerland.*

Thyroid hypertrophy, hyperplasia and neoplasia are spontaneous findings often observed in rats in non-clinical safety studies. These findings can also be induced by test compounds. To understand the time of onset, progression and potential regression of these abnormalities longitudinal (i.e., time course) assessment is important. In humans, MRI is a well established non-invasive imaging modality to examine thyroid glands for neoplasias. The purpose of this study was to back-translate this modality to the rodent model and image the rat thyroid (and parathyroid) gland by in vivo MRI. Drug-naive old male Sprague-Dawley rats were used as a model of age-related spontaneous abnormalities. Freely breathing animals were anesthetized with isoflurane. MR images of the thyroid glands were acquired before and after intravenous injection of 0.4mg/kg GdDTPA through a tail vein catheter. In vivo MRI in rats was carried out on a Bruker Biospec 4.7T/40cm instrument, equipped with a transmit body resonator and receive-only surface coil that covered the size of the animals' throat. Sagittal scout images were obtained with a fast gradient-echo sequence and used for further scan planning. Transversal high-resolution images of the thyroid glands in situ were then acquired with a RARE sequence with TR/TEeff=1530/33ms, 15 consecutive slices with 0.5mm thickness, a field-of-view of 35x35mm2, a data matrix of 256x256 points and 13 minutes scan time. After imaging, the animals were terminated, and serial sections of the thyroid glands were histopathologically examined and compared to the MRI images. Results: MRI yielded excellent tissue contrast in the thyroid (and parathyroid) gland, thus providing the prerequisite for routine and repeated volumetry of thyroid (and parathyroid) glands in situ. Comparison to histopathological sections revealed good correlation for lesions larger than approximately 500 μm in diameter. Smaller lesions (e.g., small adenomas, hyperplasia and cysts) escaped unequivocal detection by MRI.

PS 1424 INTERPRETATION OF NHANES VOC BLOOD DATA USING HEALTH-BASED SCREENING VALUES.

L. L. Aylward[1], B. Blount[2], D. Pyatt[3], C. Kirman[4] and S. Hays[5]. [1]*Summit Toxicology, LLP, Falls Church, VA,* [2]*Centers for Disease Control and Prevention, Atlanta, GA,* [3]*Summit Toxicology, LLP, Superior, Co.,* [4]*Summit Toxicology, LLP, Orange Village, OH and* [5]*Summit Toxicology, LLP, Lyons, CO.*

The National Health and Nutrition Examination Survey (NHANES) measured concentrations of 29 volatile organic compounds (VOCs) and four trihalomethanes (THMs) in blood in the general US population sampled in 2003-2004. The reported blood concentrations are evaluated in light of recently-published screening values derived from the integration of a generic steady-state PBPK model for VOCs with existing chronic exposure guidance values based on health risk assessments for both cancer and non-cancer endpoints. The most frequently detected compounds were benzene, toluene, ethylbenzene, and xylene (BTEX compounds); methyl-tert-butyl ether; 1,4-dichlorobenzene; and the THMs. Population median blood levels did not exceed non-cancer screening values for any of these compounds; upper percentile levels of benzene and xylene in blood exceeded non-cancer screening values in smokers. Population median concentrations exceeded screening values associated with 1E-05 cancer risk-specific exposure levels for benzene and bromodichloromethane, with the benzene results driven by levels in smokers. Fifteen of the 33 analyzed compounds were non-detectable in 95% or more of the sampled population. The limits of detection (LODs) employed in the analyses were generally sensitive enough to detect blood concentrations of interest based on non-cancer risk assessments, but screening values based on cancer risk-specific exposures at the 1E-05 or lower risk levels were frequently below the LODs achieved in the survey. Interpretation of blood levels of highly transient compounds in random population samples requires consideration of the likely temporal variability in blood concentrations within individuals, and the degree to which population variability may reflect or serve as a surrogate for such temporal variability. Interpretation of levels of BTEX biomarkers must take into account smoking as a major contributing source of measured levels.

PS 1425 THE UTILITY OF NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AND KIDNEY INJURY MOLECULE-1 FOR DETECTION AND SCREENING OF NEPHROTOXIC AGENTS IN PRECLINICAL DRUG DEVELOPMENT.

D. Kitchel, D. Ackley, A. McGuinty, T. Magee, W. J. Raegan and J. Aubrecht. *Drug Safety Research and Development, Pfizer Inc., Groton, CT.*

Acute kidney injury (AKI) is a rapid loss of kidney function and has a variety of causes including treatment with certain antibiotics e.g. aminoglycosides, colistin, etc. This is an important issue in clinical settings and also for screening of compounds during preclinical drug development. AKI, whether through ischemic or drug-associated causes, is clinically diagnosed mainly by a rise in serum creatinine (SCr) and blood urea nitrogen (BUN) levels as well as detection of protein in the

urine. Novel urinary biomarkers have been proposed to localize the damage to the proximal and or distal tubules vs. the glomerulus as well as to serve as an earlier indicator of kidney damage than SCr and BUN. Renal urinary biomarkers specific to tubular damage include kidney injury molecule - 1 (Kim-1) and neutrophil gelatinase-associated lipocalin (NGAL). The purpose of this study was to use Kim-1 and NGAL urine measurements in rats and canines to screen compounds for clinical use and to identify compounds that were less nephrotoxic. Kim-1 and NGAL results were compared to the more classical markers of renal injury including N-acetyl-glucosaminidase, SCr, BUN and urine protein. Animals were dosed for 2, 7 or 15 days at low and high doses with different compounds. Urine was collected at pre and post dose time points and tested for Kim-1 (rats only), NGAL, creatinine, total protein and N-acetyl-glucosaminidase. The results of Kim-1 and NGAL measurements in urine were compared to the other parameters and significant differences in results were detected between the compounds. These results combined with histopathological examination provide more comprehensive and optimal assessment of nephrotoxicity effects for drugs being developed.

PS 1426 SERUM LEVELS OF THE PHOSPHOLIPID BIS(MONOACYLGLYCEROL) PHOSPHATE AS AN INDICATOR OF PHOSPHOLIPIDOSIS INDUCED IN THE RAT BY CORALGIL.

K. Thompson[1], K. Haskins[1], N. Hartman[2], B. Rosenzweig[1], S. Stewart[1], D. Peters[1], D. Mans[3] and T. Colatsky[1]. *[1]DAPR, CDER, U.S. FDA, Silver Spring, MD, [2]LCP, CDER, U.S. FDA, Silver Spring, MD and [3]DPA, CDER, U.S. FDA, St. Louis, MO.*

Phospholipidosis (PLD) is a lipid storage disorder that can be induced in preclinical studies by more than 250 approved or investigational drugs and is characterized by the excessive accumulation of phospholipids in tissues. The incidence of PLD in patient populations is unknown due to the absence of a qualified accessible biomarker. A promising non-invasive biomarker of PLD is the rare phospholipid bis(monoacylglycerol) phosphate (BMP). The relationship between tissue incidence of PLD and BMP levels in biofluids was investigated using the drug Coralgil, which induces hepatic PLD in both patients and laboratory animals. Coralgil (4,4'-diethylaminoethoxyhexestrol dihydrochloride) was synthesized at the FDA and administered by gavage at 25, 50, or 100 mg/kg/day to male Fisher rats for 1 or 2 weeks. The drug induced a dose and time dependent increase in vacuolation in liver, lung, spleen, and mesenteric lymph node that was confirmed as PLD by electron microscopy and Lamp-2 immunohistochemistry. Liver PLD coincided with the accumulation of high levels of Coralgil and two of its metabolites in liver and with an increase in the hepatic expression of genes involved in lipid synthesis. Twelve species of phospholipid were measured in urine and serum samples. Dose related increases in serum levels of di-20:6-BMP and phosphatidylcholine species were observed after 1 week of treatment. In contrast, levels of di-20:6-BMP in urine were nonspecifically elevated in the high dose group at 2 weeks but not significantly changed at earlier times or lower doses. In this study, an increase in serum levels of BMP was an early indicator of the development of a generalized PLD induced in the rat by oral administration of Coralgil.

PS 1427 INVESTIGATION OF PREDICTIVE GASTROINTESTINAL TOXICITY BIOMARKERS FOR ONCOLOGY DRUG DEVELOPMENT IN RATS.

W. Huang[1], W. Scott[1], M. Twamley[1], E. Kindt[2], A. Wu[2], A. Sacaan[1], A. Vitsky[1] and A. John-Baptiste[1]. *[1]Drug Safety, Pfizer Global Research and Development, San Diego, CA and [2]PDM, Pfizer Global Research and Development, San Diego, CA.*

A known side effect of anti-proliferative cancer drugs is gastrointestinal (GI) injury. Early detection of GI toxicity using sensitive, predictive and reliable biomarkers would be invaluable for drug candidate safety profiling and dose selection. In this study, plasma L-citrulline, diamine oxidase, fecal calprotectin and fecal bile acids were evaluated in animal GI toxicity models induced by anti-cancer drugs with well-known but different mechanism of action. Small molecules inhibiting protein kinase CHK1, PAK4 and heat shock chaperone protein HSP90 were given at different dose levels via oral route for five days to male Wistar Han rats. Clinical signs observed were soft and watery feces, decreased skin turgor and activity, and hunched posture. Sections of the GI were collected at termination for histological and morphological evaluation, with H&E and NBT staining, respectively, to confirm GI injury. Blood and fecal samples were collected at various time points for measuring potential GI toxicity biomarkers. There was a dose- and time-dependent decrease in plasma L-citrulline levels during the 5 day study for all the compounds tested. The animals dosed with the PAK4 inhibitor (15 mg/kg) showed the most significant decrease (90%) in L-citrulline, compared to a significant decrease (75%) by CHK1 inhibitor (200 mg/kg) and a moderate decrease (35%) by HSP90 inhibitor(30mg/kg). Pathologically, rats treated with CHK1 inhibitor exhibited dose-dependent gastric ulceration, hemorrhage, and necrosis affecting the nonglandular stomach. Treatment with HSP90 or PAK4 inhibitors resulted in dose-dependent gastrointestinal lesions, with more severe, extensive findings in animals administered the latter. In conclusion, blood L-citrulline correlates with clinical observations, morphological and histological changes in both incident and severity of GI injuries and appears to be a sensitive and early predictive biomarker of GI toxicity for a variety of anti-proliferative agents.

PS 1428 EVALUATION OF A TEST METHOD FOR THE MEASUREMENT OF THE URINARY BIOMARKERS S-BENZYLMERCAPTURIC ACID AND S-PHENYLMERCAPTURIC ACID.

C. B'Hymer and J. E. Snawder. *BHAB, NIOSH, Cincinnati, OH.*

High-Performance Liquid Chromatography-Mass Spectrometric (HPLC-MS) conditions were developed for use to detect and quantitate urinary levels of S-benzylmercapturic acid (S-BMA) and S-phenylmercapturic acid (S-PMA). S-BMA and S-PMA are the metabolites from toluene and benzene, respectively, and these compounds are important for occupational health studies owing to their use as biomarkers of exposure. Toluene is used extensively as a solvent, and the health hazards of benzene exposure have been well established. The developed test method included the use of the deuterated analog of both analytes for use as internal standards. Spiked urine specimens were passed through solid-phase extraction cartridges; then the analytes were removed by acetone washes. The liquid volume was reduced by evaporation, and the dry extracts were reconstituted in mobile phase solvent for introduction into the liquid chromatograph. A triple quadrupole mass spectrometer (MS/MS) was used as detector with gradient reversed-phase HPLC conditions to measure the target analytes. Recovery experiments using 1, 2, 6, 8 and 30 ng/ml S-BMA and S-PMA fortified urine samples demonstrated good accuracy and precision. Recovery of both target analytes varied between average values of 99 to 110% of theory for the various spiked levels. Linearity of response was verified for concentrations of 0.5 to 50 ng/ml, and correlation coefficients of 0.98 or greater were obtained for all standard curves. This method offers a valid test for the determination of the urinary levels of both of these biomarkers of exposure as demonstrated by the accuracy and precision of the recovery studies.
Disclaimers: Mention of company names and/or products does not constitute endorsement by the National Institute for Occupational Safety and Health (NIOSH). The findings and conclusions in this poster have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

PS 1429 MIR-34A EXPRESSION WAS UP-REGULATED BY SEVEN DIFFERENT GENOTOXIC AGENTS IN A DOSE-DEPENDENT MANNER IN TK6 CELLS.

Z. Li, J. Yan and T. Chen. *Division of Genetic and Molecular Toxicology, U.S. FDA-NCTR, Jefferson, AR.*

MicroRNAs (miRNAs) are a class of small RNAs and play an important role in carcinogenesis. miR-34a is a tumor suppressor miRNA and its expression is directly controlled by the p53 gene to responds to DNA damage. In this study, alternation of miR-34a expression by genotoxins was determined with TaqMan real-time PCR methods to evaluate whether this miRNA could be used as an indicator for genotoxic damage. To determine the sampling time for the miRNA expression, a preliminary study was conducted. TK6 cells were treated with N-ethyl-N-nitrosourea (ENU) for 1, 2, 4, 8, 24 and 48 hours and miR-34a expression at the different time points was determined. miR-34a expression increased steadily over time and reached the highest at the 24 hour point. For the main experiment, TK6 cells were treated with 7 genotoxins: ENU, cisplatin, etoposide, mitomycin C (MMC), methyl methane sulfonate (MMS), toxal and NaCl at low, medium and high doses for 4 hours. Each treatment was in triplicate and the high doses were based on 50% cytotoxicity (relative growth rate). After the treatment, the cells were washed and cultured for additional 20 hours, and then their miR-34a expression was determined. The miR-34a expression was significantly up-regulated by each of the genotoxins in a dose dependent manner. The expression changes for the low, medium and high doses were 1.5-, 4.5-, and 5.9- fold for ENU; 1.6-, 2.8 and 7.1-fold for

cisplatin; 1.2-, 1.9- and 2.9-fold for etoposide; 1.5-, 3.1-, and 6.0-fold for MMC; 1.7-, 3.1- and 3.8-fold for MMS; 2.2-, 3.7- and 5.0-fold for taxol; and 1.1-, 2.8- and 3.3-fold for NaCl, respectively. The results demonstrate that miR-34a responds to genotoxic insults sensitively and dose-dependently. Therefore, it has the potential to become a biomarker for exposure of genotoxic carcinogens.

PS 1430 VALIDATION OF DIKI BIOMARKERS IN CYNOMOLGUS MONKEY.

M. Guha[1], A. Heier[4], S. Price[4], M. Balenstein[5], R. Caccese[1], T. Simpson[2], D. Stong[1], L. Kinter[1], Y. Dragan[1] and E. Bodes[3]. *[1]Safety Assessment, Astra Zeneca, Wilmington, DE, [2]Medicinal Chemistry, AstraZeneca Pharmaceuticals, Wilmington, DE, [3]Emerging Product Team, AstraZeneca Pharmaceuticals, Wilmington, DE, [4]Safety Assessment, AstraZeneca Pharmaceuticals, Alderly Park, Manchester, United Kingdom and [5]Clinical Pharmacology &DMPK, AstraZeneca Pharmaceuticals, Sodertalje, Sweden.*

Drug-induced kidney injury (DIKI), assessed by histopathology, results in attrition in drug development; new DIKI urinary biomarkers offer potential to detect and monitor DIKI progression and regression, but currently only in rats. The triple reuptake inhibitor PRC200 represents a new class of antidepressants that elevate synaptic levels of serotonin, norepinephrine and dopamine, and are expected to produce more rapid onset and better antidepressant efficacy than single or dual inhibitors. Using histopathology, DIKI biomarkers, and toxicokinetic assessments in single (25-50 mg/kg) and repeat (10-25 mg/kg/day for 28 days) dose toxicological studies in cynomogolus monkeys (NHP), we characterized dose proportional DIKI associated with oral administration of PRC200. Histologically, degeneration and regeneration of kidney tubules associated with inflammation were present. Using a newly developed triple immunohistochemistry (IHC) method, with antibodies against calbindin D28, aquaporin2, and aquaporin1, we detected selective injury of distal tubules (DT) and collecting ducts (CD). DIKI biomarker analyses, using multiplex immunoassays, of urine samples confirmed dose proportional increases in the excretion of calbindin D28 in treated monkeys with levels returning to baseline during the drug free recovery period. In addition, elevated clusterin levels, during treatment and recovery periods, in urine correlated with the histopathology of the kidneys and supported the IHC observations. These results constitute an early, if not the first validation of distal nephron DIKI biomarkers in NHP and demonstrate the utility of calbindin D28 and clusterin to monitor the progression of distal nephron DIKI in this important non-rodent preclinical species with a potential application in the clinic.

PS 1431 SHIPPING OF NONCLINICAL SPECIMENS: THE IDEAL CONDITION FOR CONSISTENCY OF SAMPLE HANDLING THROUGHOUT THE DRUG DEVELOPMENT PROCESS.

J. J. Stewart, D. Efterfield, A. Powell and L. Brown. *Flow Contract Site Laboratory, LLC, Kirkland, WA.* Sponsor: J. Hill.

Rationale: A wide variety of specimen types, including whole blood, peripheral blood mononuclear cells (PBMC) and various tissues can be analyzed by flow cytometry for drug development studies. When shipping samples to a laboratory for analysis, cell integrity, morphology and antigenic site activity can be compromised due to unfavorable environmental conditions and delivery delays. However, shipping of samples has many benefits such as an increase in choice of testing laboratories, improved consistency using the same laboratory for non-clinical as well as clinical analysis and maximizing the use of laboratories that specialize in specific types of testing, such as flow cytometry. Experimental Procedures: In our study we tested non-human primate (NHP) whole blood specimens shipped overnight in Cyto-Chex® BCT (blood collection tubes) and compared the results to more traditional anticoagulants such as Na Heparin and EDTA. Whole blood immunophenotyping of surface and intracellular markers was evaluated by flow cytometry on a BD FACSCanto™ equipped with BD FACSDiva™ software. Rodent tissue specimens were shipped in 100% FBS and the resulting cell suspensions were evaluated for cell count and viability on a Guava® PCA equipped with Cytosoft™ software. Results: Our study showed that NHP whole blood specimens can keep their integrity for 24-48 hours using EDTA tubes, 72 hours using Na Heparin tubes and up to one week using Cyto-Chex® BCT when held at room temperature. For the tissue specimens, viability and cell count can be directly impacted by shipping temperature. Shipping tissue specimens on frozen gel packs caused a decrease in viability (50.7–72.2%) compared to refrigerated gel packs (82.2–91.1%). Conclusion: Despite the risks of shipping, monitoring shipping temperature and use of a stabilizing anticoagulant that helps maintain whole blood sample integrity for longer periods can make shipment of non-clinical samples more reliable and allow for greater flexibility in the choice of flow cytometry laboratory.

PS 1432 VALIDATION OF BIOMARKERS FOR ASSESSMENT OF CARDIAC EFFECTS IN RATS.

J. C. Mirsalis, N. Chini, D. Vuong, K. Altera, B. Schneider and H. Ng. *Preclinical Development, SRI International, Menlo Park, CA.*

Biomarkers of target organ toxicity have recently emerged that can dramatically improve safety predictions. To assess the utility of cardiac biomarkers used in rodent toxicology studies, male SD rats were administered a single subcutaneous (sc) injection of isoproterenol (IPT; 5 or 10 mg/kg), hydralazine (HDZ; 25 mg/kg), doxorubicin (DOX; 20 mg/kg) or saline. Blood samples were collected 4 hr, 48 hr and 7 days after dosing. Blood was evaluated with a troponin I (TNI) Triage cardiac kit and the Mesoscale Discovery (MSD) muscle injury kit (cTnI, cTnT, FABP3, Myl3, sTnI). Rats (5/group/time) were euthanized 48 hr and 7 days after dosing. Hearts were evaluated histopathologically. Cardiac lesions seen at both evaluation times following IPT or HDZ treatment included myocyte degeneration/necrosis, atrophy and vacuolation; non-suppurative inflammation; fibrosis; endocarditis; epicarditis; and edema. Cardiac lesions were not observed with DOX treatment. IPT (5 mg/kg) produced significant increases in cardiac biomarkers: TNI, cTnI, cTnT, FABP3 and Myl3 were increased 84-, 721-, 28-, 4- and 38-fold, respectively, over controls 4-hr after dosing. Levels dropped dramatically by Day 3, with no effects on TNI and cTnI, and 3.5-, 2.3-, and 9.8-fold increases for cTnT, FABP3, and Myl3, respectively. HDZ showed similar effects with 43- and 37-fold increases at 4 hr post dose for TNI and cTnI, respectively, but only modest increases in other parameters. DOX had negligible effects on cardiac markers at 4 hr, but had a 21-fold increase in sTnI (skeletal troponin); Myl3 was 9.8-fold higher at 48 hr and 7.4-fold higher on Day 8. Stability testing of frozen samples indicated that cTnI and sTni remain within 10% of original values over 4 months, whereas cTnT, FABP3, and Myl3 give values ranging from 24-61% higher. These results indicate that cardiac biomarkers are highly effective at identifying early cardiac changes that are predictive of cardiotoxicity, and are a valuable addition to conventional clinical pathology indicators of toxicity. Work supported by NIAID Contract N01-AI-70043.

PS 1433 INSTABILITY OF QUINONE ELECTROPHILE ADDUCTS ON CYSTEINE RESIDUES: A BASIS FOR THE PREFERENTIAL DETECTION OF COVALENT MODIFICATION OF LYSINES AND ARGININES.

C. L. Kuhlman[1], M. L. Labenski[1], A. A. Fisher[1], B. Z. Olenyuk[2], T. J. Monks[1] and S. S. Lau[1]. *[1]Southwest Environmental Health Sciences Center, Department of Pharmacology/Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ and [2]Department of Chemistry/Biochemistry, College of Science, University of Arizona, Tucson, AZ.*

Quinones represent an important class of electrophilic xenobiotics that have known reactivity based on their ability to redox cycle and generate oxidative stress, and to alkylate proteins. In vitro and in vivo studies reveal lysine and arginine residues to be primary targets of 1,4 Benzoquinone (BQ) adduction. Though cysteine residues are commonly reported targets of electrophilic adduction, we were unable to detect such adducts with BQ. Reaction of a cysteine-containing peptide (acAQGSCGPNLSOH) with BQ, and subsequent MALDI-TOF and LC-MS/MS analysis confirmed that BQ does indeed adduct cysteine residues. However, replicating a standard protein digestion protocol at pH 7.5 with 100 mM ammonium bicarbonate or 50 mM Tris-HCl confirms that cysteine-BQ adducts within acAQGSCGPNLSOH are unstable. Monitoring the peptide-BQ adducts via consecutive HPLC analyses revealed that BQ-cysteine adducts are degraded within 1 hr. BQ-adduct stability was further investigated on cytochrome C, which upon heme removal has two cysteines free and available for protein adduction. BQ adducts within apocytochrome c were similarly unstable in standard protein digestion buffer conditions. Taken together, the results indicate that BQ-adduct formation on cysteine residues is a transient interaction, where physiological conditions dictate adduct stability. Thus, quinone post-adduction chemistry ex vivo plays a critical role in determining the ability to identify amino acid targets of quinones. Lysine and arginine residues on proteins may be preferential targets for covalent adduction by quinones under physiological conditions, providing more stable adducts for mass spectral analysis, a prerequisite for useful biomarker identification under normal physiological conditions. (GM007890, P30ES006694, Sci. Found. of AZ, T32ES007091)

PS 1434 URINARY BIOMARKER DETECTION OF MELAMINE-CYANURIC ACID-INDUCED KIDNEY DAMAGE IN RATS.

Q. Zhang[1], G. Gamboa da Costa[2], R. Beger[2], L. Schnackenberg[2], J. Sun[2], L. Pence[2], S. Bhattacharyya[2], C. Jacob[2], R. P. Brown[1] and P. L. Goering[1]. [1]*CDRH, U.S. FDA, Silver Spring, MD and* [2]*NCTR, U.S. FDA, Jefferson, AR.*

Exposure of rats to melamine and cyanuric acid in the feed can result in a dose-dependent increase in formation of melamine-cyanuric acid crystals in the kidney. The aim of this study was to determine if urinary biomarkers of acute kidney injury can be used to detect noninvasively renal damage associated with crystal formation in the kidneys of melamine-cyanuric acid-exposed rats. Male and female Fischer 344 rats were fed a melamine-cyanuric acid mixed diet at approximately 5, 10 and 15 mg/kg body weight/day for 28 days. Urine samples were collected at 1, 2, 3, 4, 14, and 28 days. A series of urinary protein biomarkers (Kim-1, NGAL, osteopontin, alpha-GST, GSTYb1, RPA-1 and clusterin) were measured by MesoScale Discovery multiplex assay system. Urinary metabolites were evaluated by NMR and LC/MS metabolomic assays. The results showed that RPA-1 (distal tubule and collecting duct injury biomarker) was elevated at the 10 and 15 mg/kg doses in a time-dependent manner; however, there were no changes observed in other urinary protein biomarkers at any other dose or time point. Metabolomic analysis showed increased levels of urinary proline on days 3 and 28 in male rats and day 28 in female rats at the 15 mg/kg dose only. An increase of urinary hydroxyproline was observed on day 28 in male rats and on day 1 in female rats at 15 mg/kg. Significant renal histopathological changes were observed at the 15 mg/kg dose, although variable levels of melamine-cyanurate crystal formation were observable at the lower dose levels. These data indicate that increased levels of urinary RPA-1 and various urinary amino acids are seen at doses of melamine-cyanuric acid that produce histopathological damage in the kidney. As a result, these endpoints may serve as promising noninvasive urinary biomarkers for the detection of acute kidney injury associated with melamine-cyanuric acid exposure.

PS 1435 VALIDATION OF BEST DETECTION METHODS FOR MEASURING OXIDATIVE STRESS.

M. B. Kadiiska. *NIEHS/NIH, Research Triangle Park, NC.*

Many techniques now exist that potentially allow the measurement of oxidative stress in experimental animal models and humans. The concept of measuring oxidative stress status in humans has not been approached as a real possibility in clinical practice because standardized methods are not yet established. NIEHS/NIH has taken the lead in organizing and sponsoring the first national and international comprehensive comparative study for determining which of the available biomarkers of oxidative stress are most specific, sensitive and selective. Investigators from 25 laboratories are participating in this study, called BOSS. The goal of the BOSS is to validate and find the best markers of oxidative stress that are applicable to different oxidative insults and measurable in stored specimens. Antioxidants and various oxidative products of lipids, proteins and DNA were measured in blood, plasma and urine of experimental animals with three different oxidative insults of CCl4 poisoning, ozone exposure, and LPS treatment. It was found that plasma concentrations of MDA and F2-isoprostanes (measured by GC/MS) and urinary concentrations of isoprostanes (measured with ELISA) were increased in CCl4- and ozone-exposed rats, and LPS-treated mini pigs in a time- and dose-dependent manner. Antioxidants (GSH/GSSG, tocopherols, ascorbic acid, uric acid, and total antioxidant capacity), oxidation products of proteins (protein carbonyls, methionine-sulfoxidation, and tyrosine oxidative products) and DNA (strand breaks, 8-OH-dG, M1G) were not significantly changed. It is concluded that measurements of plasma lipid peroxidation products MDA and isoprostanes (by GC/MS) and urinary isoprostanes (by ELISA or GC/MS) are promising candidates for general biomarkers of oxidative stress. Various antioxidants, protein- and DNA-oxidation products are not identified as biomarkers of oxidative stress induced by CCl4-, ozone- or LPS- exposures.

PS 1436 STEROID HORMONE PROFILING BY MASS SPECTROMETRY IN RAT BLOOD COLLECTED *VIA* THE CULEX® AUTOMATED *IN VIVO* SAMPLING SYSTEM.

C. Penno[1, 2], L. Morawiec[1], M. Schwald[1], N. Zamurovic[1], S. Busch[1], A. Wolf[1], F. Pognan[1], S. Chibout[1], A. Odermatt[2] and M. Dong[1]. [1]*Preclinical Safety, Novartis, Basel, Switzerland and* [2]*Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland.*

Impaired steroid hormone regulation has been implicated in the pathogenesis of multitudinous drug-induced toxicities. A quantitative profile of steroid hormone metabolites holds great potential to help assessing drug safety, dissecting toxic mechanisms, understanding disease progress and providing rational therapeutic intervention. A novel LC-MS/MS method has been established to quantify a panel of 12 steroid hormones: aldosterone, cortisol, cortisone, corticosterone, 11-dehydrocorticosterone, 11-deoxycorticosterone, 11-deoxycortisol, testosterone, dihydrotestosterone, androstenedione, 17α-hydroxyprogesterone and progesterone (comprising rodent and human metabolites). Steroid metabolites were resolved by liquid chromatography using an Allure Biphenyl (Restek) column and quantified by Agilent 6410 Triple-Q mass spectrometer. The method presented excellent dilution linearity for all 12 metabolites and the limits of quantitation were ranging from 250 pM for androstenedione to 4 nM for corticosterone. Method validation and measurements were performed in 50 μL of rat plasma spiked with calibrators and deuterized internal standards. The method was then applied to profile time-course changes of steroid hormones in rat plasma. Wistar male rats were connected to the Culex® system through carotid artery or jugular vein. Blood collection was programmed every 3 hours during a 24-hour period. Interim results indicated that changes of corticosterone, 11-dehydrocorticosterone, and 11-deoxycortisol followed the known circadian rhythm, suggesting the non-disturbance of glucocorticoid profiles in catheterized rats. Levels of testosterone, androstenedione and progesterone were rather constant regardless of the day and night shift. The other steroid metabolites analyzed were below the detection limit. Samples from additional animals are currently being analyzed to gain a high level of statistical power.

PS 1437 SERUM CARDIAC TROPONIN I CONCENTRATION AS AN EARLY BIOMARKER OF MYOCARDIAL HYPERTROPHY IN THE ROSIGLITAZONE OR 3, 3', 5-TRIIODO-L-THYRONINE TREATED RAT.

M. Dunn[1], D. Coluccio[1], R. Fernandes[1], D. Knapp[1], R. Nicklaus[1], G. Hirkaler[1], W. Geng[1], M. Sanders[1], T. Singer[2] and I. Mikaelian[1]. [1]*Nonclinical Safety, Roche, Nutley, NJ and* [2]*Nonclinical Safety, Roche, Basel, Switzerland.* Sponsor: K. Kolaja.

Background: The peroxisome proliferator-activated receptor gamma (PPARγ) agonist rosiglitazone has been linked to cardiac hypertrophy in rats and an increased risk of myocardial infarction in man. Exclusive to the heart, cardiac troponin (cTn) I is released in response to myocardial damage. Our goal was to investigate the link between cardiac hypertrophy and low level changes of serum cTnI concentrations following rosiglitazone or 3,3',5-triiodo-L-thyronine (T3) treatment in the rat. Methods and Results: Serial blood samples from male rats were evaluated following dosing of rosiglitazone (80 mg/kg/day) or T3 (20 μg/kg/day) for 14 or 28 days, with 14 or 27 days of recovery. Histopathology and clinical chemistry, including measurement of cTnI concentrations with the Singulex Erenna® Ultrasensitive Immunoassay were performed. Compared to aged-matched vehicle treated controls, a treatment-duration dependent increase of heart weight to brain weight was observed in both rosiglitazone (10% and 17%) and T3 (21% and 30%) treated groups after 14 and 28 days of treatment, respectively, and this cardiac hypertrophy was found to be reversed after the recovery period. Across treatment groups and at all time points, a positive correlation was established between cTnI concentration and the degree of cardiac hypertrophy. Significant elevations of cTnI were quantified after 14 and 28 days of rosiglitazone treatment, in the absence of histomorphologic findings. As expected, T3 treatment induced marked and progressive elevations in cTnI concurrent with histiocytic infiltration of the myocardium after 14 and 28 days of dosing, and necrosis after 28 days of dosing. Conclusion: Our results demonstrate the sensitivity of low level cTnI concentration changes as indicative of myocardial hypertrophy which may occur in the absence of histomorphologic findings. These results lend support for serum cTnI concentrations as an early biomarker of cardiovascular liability.

PS 1438 CHARACTERISATION OF THE PRODROMAL AND REVERSIBILITY VALUE OF NOVEL RENAL SAFETY BIOMARKERS IN GENTAMICIN-TREATED RATS.

J. Wood[1], A. Shoieb[1], A. McGuinty[1], P. Cleall[1], D. Hardy[1], D. Collins[1], C. Dubray[1], R. Williams[1], A. Moody[1], K. Lynch[2], D. Ennulat[2], T. Sellars[2], T. Lambert[2], S. Beushausen[3], E. Harpur[4], M. Guffroy[1] and D. Brees[1]. [1]*Pfizer, Sandwich, United Kingdom,* [2]*GlaxoSmithKline, King of Prussia, PA,* [3]*Pfizer, St. Louis, MO and* [4]*Sanofi-Aventis, Alnwick, United Kingdom.* Sponsor: D. Robinson Gravatt.

The objectives of this ILSI-HESI sponsored study were to further define the value of the novel renal biomarkers during the prodromal and reversibility phases of renal injury, using Gentamicin as a test toxicant. Gentamicin was administered by subcutaneous injection once daily to groups of 10 male Sprague-Dawley rats at 50 mg/kg/day for 1, 4 or 10 days. Overnight urine samples were collected pre-dose (days -7 and -3), on Days 2, 5, 11 (dosing period), and on Days 18, 25, 32 and 39 (recovery period) and analysed for Kim-1, NGAL, Osteopontin, Albumin, Regucalcin, Beta-2-Microglobulin, Cystatin C, Clusterin, GST-α, GST-Yb1, RPA-1, NAG, Creatinine and Protein. Microscopically, tubular epithelial degeneration/necrosis of the proximal convoluted tubules was observed on Day 5, peaked on

Day 11 and started to recover on Day 18. These changes correlated with statistically significant increases (p<0.01) in Albumin, Beta-2-Microglobulin and Clusterin by Day 2, in all biomarkers with the exception of Kim-1, Regucalcin and RPA-1 by Day 5, and in all biomarkers, including serum creatinine and BUN, by Day 11. In the absence of degeneration/necrosis on Day 2, biomarkers elevated at this time point could be considered as prodromal markers of kidney injury. Basophilic tubules consistent with regeneration were observed microscopically as early as Day 5, but were most pronounced on Day 18. The only biomarker which correlated with these changes was Osteopontin, where levels were most elevated on Day 18. Osteopontin could therefore be considered as a biomarker of regeneration. Reversibility was evident microscopically by Day 39, but was not complete. Although all biomarkers were decreased during the recovery phase, Kim-1, Osteopontin and Cystatin C did not recover fully. This suggests that these biomarkers may be the most sensitive at tracking residual injury and ongoing renal changes.

PS 1439 EVALUATION OF NOVEL BIOMARKERS OF NEPHROTOXICITY IN MICE.

J. Gautier[1], T. Gury[1], J. Léonard[1], O. Dorchies[1], L. Stimmer[1], E. Harpur[2], S. Küsters[3], J. Mapes[3] and M. Guffroy[1]. *[1]sanofi-aventis, Paris, France, [2]sanofi-aventis, Alnwick, United Kingdom and [3]Rules-Based Medicine, Austin, TX.*

Most studies to evaluate renal safety biomarkers have been performed in rats. This study was conducted in mice to evaluate the potential usefulness of novel biomarkers of nephrotoxicity for different segments of the nephron in this species.
Adult mice were given daily 30, 100, 300 mg/kg/day for 14 days with a compound under preclinical development previously shown to induce renal toxicity. Blood samples and 16-hour urine samples were collected at the end of dosing period on Day 15 at all doses and following a 2-week recovery period on Day 29 at 300 mg/kg/day. Urinary parameters evaluated (after normalization to urinary creatinine) included NAG, microalbumin, calbindin, clusterin, cystatin C and Kim-1. Calbindin, clusterin and cystatin C were measured using luminex bead-based rat assays, and Kim-1 with a newly developed mouse-specific assay. Kidney microscopic findings on Day 15 consisted in partially reversible degeneration/necrosis and regeneration of proximal tubules from 100 mg/kg/day, and of distal segments of the nephron (thick ascending limbs of Henle's loops, collecting ducts) at 300 mg/kg/day. The localization of the kidney lesions was confirmed by immunohistochemistry. There were no increases in BUN and serum creatinine at all dose levels. When compared to mean control values, moderate to marked dose-related and reversible increases in mean urinary microalbumin (up to 62-fold), cystatin C (up to 21.3-fold), calbindin (up to 17.1-fold) and clusterin (up to 6.0-fold) were observed at 100 and 300 mg/kg/day. Mild reversible increases in mean urinary NAG (up to 3.9-fold) and Kim-1 (up to 8.5-fold) were observed at 300 mg/kg/day.
These results indicate that the urinary proximal tubular biomarkers microalbumin, cystatin C, NAG, Kim-1, together with the distal nephron segment biomarker calbindin, and the non specific segment biomarker clusterin may be useful biomarkers to monitor kidney lesions in mice and potentially in humans in the absence of increases in BUN and serum creatinine.

PS 1440 IMPACT OF NUTRITION ON THE PLASMA METABOLOME IN RATS.

W. Mellert[1], M. Kapp[1], V. Strauss[1], J. Wiemer[2], E. Leibold[1, 2], H. G. Kamp[1, 2], T. Walk[2], R. Looser[2], A. Prokoudine[2], E. Fabian[1], G. Krennrich[1], M. Herold[2] and B. van Ravenzwaay[1]. *[1]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany and [2]Metanomics GmbH, Berlin, Germany.*
Sponsor: R. Landsiedel.

Metabolite profiling (analysis of endogenous low molecular compounds in plasma) is a relatively new technology to elucidate changes in biochemical pathways under various condition (e.g. different nutrition scenarios or after administration of test compounds). BASF and metanomics have obtained the metabolic profiles of approximately 500 compounds (agrochemicals, chemicals and pharmaceuticals) in rats. With these profiles we were able to establish specific metabolic patterns associated with a very large number of toxicological modes of action (the MetaMap®Tox database). To elucidate possible confounding factors which may influence the outcome of metabolome patterns, we studied the effect of fasting vs. non-fasting prior to blood sampling. In addition, we evaluated the influence of high caloric diet and caloric restriction as well as the administration of corn oil and olive oil as vehicles on the metabolome of male and female rats. All of the treatments mentioned above had a distinct effect on the metabolome. Changes in metabolites can be classified in "biomarkers of exposure" such as diet-related metabolites and "biomarkers of effect" such as energy-related metabolites. Of particular interest was a profound difference in metabolome response between male and female rats when subjected to caloric restriction. As all these treatments are associated with the nutritional status of the animals, they need to be considered in the evaluation of metabolic profiles after chemical exposure.

PS 1441 UTILITY OF MICRORNAS AS POTENTIAL BIOMARKERS OF TESTICULAR TOXICITY.

H. Lin[3], J. Milano[1], L. Barone[1], J. Stewart[2], S. Ramaiah[3] and Y. Dragan[1]. *[1]Safety Assessment, AstraZeneca Pharmaceuticals, Wilmington, DE, [2]Reproductive Toxicology, AstraZeneca Pharmaceuticals, Alderley Park, Cheshire, United Kingdom and [3]DSRD, Pfizer Inc., Cambridge, MA.*

Estimation of the toxicologic effects on male fertility is a critical point for going into the first human trial of new drug entities. Risk assessment for effects on male reproduction is based primarily on testicular histopathology. Histopathological evaluation, however, is not easily used for longitudinal monitoring nor is it translatable to man. MicroRNAs (miRNAs), a family of small non-coding RNAs involved in regulation of gonadogenesis and spermatogenesis, have emerged as novel biomarkers and potential therapeutic targets. We have previously demonstrated that the abundance of miRNAs in blood, their testicular specificities, the differential expressions with treatment of testicular toxicants. In this study we focus on dysregulated miRNAs in testes of rats treated with Sertoli toxicant, 2,5-Hexanedione (2,5-HD). We analyzed the expression of miRNAs in testes of 2,5-HD treated rats and identified significantly dysregulated miRNAs. Notably, several identified miRNAs were localized in the chromosome 6q miR-543 cluster. The miR-154 gene family was over represented among the identified miRNAs with 6 members, miR-323, miR-381, miR-487b, miR-382, miR-409, and miR-369 significantly dysregulated. Pathway analysis revealed that the predicted target genes are concentrated in select biological pathways, exemplified by reproduction FSH beta signaling pathway, cell cycle regulation, CFTR and TGF beta signaling pathways. Computational mapping of the upstream region of the intergenic miRNA cluster discovered binding sites of AP-2, an important transcription factor in testis. The concordant regulation of members of the mir-543 cluster/miR-154 family suggests that this group plays a potential role in the testicular response to a Sertoli toxicant. These results provide further support for the utility of miRNAs as potential biomarkers for assessing testicular toxicity.

PS 1442 SURFACE PLASMON-BASED CELL AND PROTEIN MICROARRAYS FOR THE IDENTIFICATION OF STRESS AND DISEASE BIOSIGNATURES.

J. M. Rice[1], C. Rich[1], P. E. Reinhold[1], D. A. Lawrence[2] and M. A. Lynes[1]. *[1]Molecular and Cell Biology, University of Connecticut, Storrs, CT and [2]New York State Department of Health, Wadsworth Center, Albany, NY.*

The emerging area of microspot array technology has a considerable potential for the meaningful identification of molecular and cellular biomarkers. We are developing microarray surface plasmon-based assays to reveal unique biosignatures of stress and disease. By employing a small sensor chip, the generated surface plasmon is utilized to make two independent measurements of ligand binding. The first of these, grating coupled surface plasmon resonance imaging (GCSPRI), allows for label-free detection of captured cells and high concentration analytes. Subsequent surface plasmon enhanced fluorescence (SPEF) measurements allow for the measurement of fluorescently tagged markers on the same sensor chip. Combining these two approaches in sequence has allowed us to achieve an approximately 8 log dynamic range of analyte detection for TNF-alpha, and femtomolar sensitivities for cytokine detection that are consistently below the limit of detection (LOD) for ELISA. The GCSPRI technology can be used as a solid phase cytometer for the quantification of influenza peptide reactive T cells using MHC monomers with a LOD of 0.1% antigen reactive cells. We have also measured on-chip leukocyte cytokine secretion in an in vivo Cd2+ exposure model as an example of combining cell detection and cytokine secretion in a single assay. The increased sensitivity afforded by SPEF also allows the analysis of low concentration biomarkers in easily obtained bio-fluid samples such as saliva. For example, elevated levels of salivary amylase, cortisol, and IgA were measured in saliva from individuals exposed to traumatic stress. These multiplexed assays should reveal unique biosignatures that can more accurately diagnose different types of stresses or toxicant exposures.

PS 1443 INITIAL BIOLOGICAL QUALIFICATION OF SBDP-145 IN CEREBROSPINAL FLUID AS A BIOMARKER OF COMPOUND-INDUCED NEURODEGENERATION IN THE RAT.

M. L. Pritt[1], W. H. Jordan[2], D. G. Hall[1], D. W. Ballard[1], U. W. Muller[3], K. W. Wang[3] and D. E. Watson[1]. *[1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, [2]Vet. Path Services, Mason, OH and [3]Banyan Biomarkers, Alachua, FL.*

Neurodegeneration is a safety risk associated with some compounds that are pharmacologically active in the CNS. However, monitoring neurodegeneration is difficult in both pre-clinical and clinical drug development. A CSF-based protein biomarker of neurodegeneration could improve non-clinical safety assessments and

our ability to monitor patient safety in clinical studies compared with reliance on brain histopathology in veterinary species and clinical observations in patients. Non-erythroid alpha-II spectrin is a cytoskeletal protein that is cleaved by the protease calpain when this enzyme is activated by an influx of calcium into injured cells. Spectrin breakdown products of 150, 145, and 120 kDa (SBDP-150, -145, and -120, respectively) increase in concentration with increased magnitude of traumatic brain injury in rats and humans. We hypothesized that similar influxes of cellular calcium that occur during compound-induced neurotoxicity would cause sufficient activation of calpain to generate SBDP's detectable in CSF, and that these SBDP's would be sensitive biomarkers of compound-induced neurodegeneration. We administered neurotoxicants (kainic acid, 2-chloropropionic acid, bromethalin, pentylenetetrazole) affecting different portions of the brain to rats in dose-response and time-course studies in which we characterized the neurodegeneration by histopathology and measured SBDP-145 concentrations in CSF. We consistently observed > 3-fold increases in SBDP-145 concentration in rats with minimal-slight lesions, and 20-150-fold increases with more severe lesions. These initial data support expanded use of SBDP-145 as a biomarker for monitoring compound-induced neurodegeneration in pre-clinical studies, and suggest potential clinical applications of this biomarker for monitoring neurodegeneration.

PS 1444 PET IMAGING OF TRANSLOCATOR PROTEIN 18 kDa (TSPO) REVEALS DELAYED NEUROINFLAMMATION COINCIDENT WITH PERSISTENT BEHAVIORAL DEFICITS IN RATS ACUTELY INTOXICATED WITH DFP.

D. A. Bruun[1], D. Rowland[2], Y. Li[1], B. Ford[3] and P. Lein[1]. [1]*Molecular Biosciences, University of California, Davis, CA,* [2]*Center for Molecular and Genomic Imaging, University of California, Davis, CA and* [3]*Anatomy and Neurobiology, Neuroscience Institute, Morehouse School of Medicine, Atlanta, GA.*

Neuroinflammation has been reported in the brains of animals that survive acute intoxication by organophosphorus (OP) nerve agents and insecticides, suggesting that neuroinflammation may be a useful biomarker of OP-induced neurotoxicity. TSPO is expressed in the brain at low levels under normal physiological states, but is markedly upregulated at sites of neuronal injury and inflammation. In this study, we evaluate PET imaging of TSPO as an in vivo biomarker of neurotoxicity following acute OP intoxication. Adult male Sprague Dawley rats administered the TSPO ligand [11C]PK11195 were imaged immediately prior to and 2, 7 and 21 days following administration of diisopropylfluorophosphate (DFP, 9 mg/kg, i.p) or an equal volume of vehicle (saline) in combination with atropine methyl nitrate (20 mg/kg, i.m.) and pyridostigmine (0.1 mg/kg, i.m.). MRI imaging was performed in the same animals to allow localization of TSPO expression within specific brain regions. PET images revealed an obvious upregulation of TSPO at 7 but not 2 or 21 days post-exposure in DFP-treated rats. Quantification of TSPO labeling in the whole brain indicated that TSPO levels in DFP-treated rats increased by 250% over baseline values at 7 days post-exposure; in contrast TSPO levels did not significantly change from baseline values in control animals over the course of the experiment. Deficits in learning and memory as determined using contextual fear conditioning were observed in DFP-exposed rats on day 9 post-exposure and these persisted until day 20 post-DFP exposure. These studies suggest the feasibility of using PET imaging of TSPO for in vivo monitoring of not only delayed neuronal damage following acute OP intoxication but also the therapeutic efficacy of medical countermeasures. Supported by the NIH CounterAct program (UO1 NS057993).

PS 1445 MOVING BEYOND THE ORTHODOX 'TBNK' PANEL: NEW IMMUNOPHENOTYPING STRATEGIES FOR IMMUNE-TOXICOLOGICAL ASSESSMENTS IN PRECLINICAL AND CLINICAL PHARMACEUTICAL STUDIES.

T. Warren, A. R. Mcintyre, T. Salewsky, R. Eyre and H. Tsusaki. *SNBL USA, Ltd., Everett, WA.*

The three to six color TBNK panel assessing T-lymphocytes, B-lymphocytes, and natural killer cells (CD3, CD4, CD8, CD16, and CD20) is a staple of the hospital clinical flow cytometry laboratory. With the new biological class of pharmaceutical drugs currently being tested in preclinical studies, the need to expand beyond three cell types and their subsets becomes apparent. There are presently over 360 human cluster of differentiation (CD) markers established by the Human Leukocyte Differentiation Antigens (HLDA) committee that are identified as cell receptors on white blood cells. Here we propose a new paradigm for immunological assessment of drug toxicity on the immune system. Immunophenotyping is performed using a six tube panel with six colors for a broad assessment of the main blood populations and extended panels that detail the individual cell subsets. The first tube assesses all mononuclear cells using the markers CD3, CD14, CD20, CD45, CD66, and CD159a. Mononuclear cell subsets are delineated using tubes for T-lymphocytes (CD3, CD4, CD8, CD25, CD28, and CD45RA), B-lymphocytes (CD19, CD20, CD21, CD27, CD40, and IgD), Natural Killer Cells (CD3, CD25, CD107a, CD159a, CD335, and CD337), Monocytes (CD3, CD11b, CD14, CD16, CD32, and CD64), and Neutrophils (CD11b, CD14, CD18, CD35,CD64, and CD66). This approach allows for extensive data mining to detect changes even in minute populations of cells, providing a thorough and robust assessment of a drug's effect on the immune system.

PS 1446 GENOMICS AND PROTEOMICS ANALYSIS IDENTIFIED THE PROTEINS ASSOCIATED WITH ADIPOCYTE LIPOLYSIS AS A KEY REGULATOR FOR THE PATHOGENESIS OR PROGRESSION OF METABOLIC SYNDROME.

J. Chang[2], S. Oikawa[4], C. Ando[1], K. Kuzuya[1], G. Ichihara[3] and S. Ichihara[2, 1]. [1]*Life Science Research Center, Mie University, Tsu, Japan,* [2]*Graduate School of Regional Innovation Studies, Mie University, Tsu, Japan,* [3]*Nagoya University Graduate School of Medicine, Nagoya, Japan and* [4]*Department of Environmental and Molecular Medicine, Mie University Graduate School of Medicine, Tsu, Japan.*

Background: It is difficult to study the mechanisms of the metabolic syndrome in humans because it attributes to the heterogeneous genetic background and lifestyle. The present study investigated the alteration of gene and protein profiles in the animal model of metabolic syndrome to find possible genes and proteins related with metabolic syndrome. Methods and Results: We extracted RNA and protein from the epididymal adipose tissue and liver in the spontaneously hypertensive/NIH - corpulent rat SHR/NDmcr-cp (cp/cp) (CP), SHR/Lean (Lean), or Wistar Kyoto rat (WKY). On the oligo DNA microarray, 221 genes in the adipose tissue and 53 genes in the liver were significantly up-regulated ($p < 0.01$) in 6 weeks-old CP compared to 6 weeks-old Lean or WKY. Moreover, 323 genes in the adipose tissue and 48 genes in the liver were down-regulated in 6 weeks-old CP compared with 6 weeks-old Lean and WKY. The 25 weeks-old CP showed significant up-regulation of 140 genes and down-regulation of 121 genes in the adipose tissue as well as significant up-regulation of 72 genes and down-regulation of 83 genes in the liver compared to 25 weeks-old Lean and WKY. We also observed differential protein expression in the adipose tissue at the early and chronic stages on metabolic syndrome using 2D-DIGE connected with MALDI-TOF-TOF analysis. Among them, the expression of monoglyceride lipase is significantly increased and carboxylesterase 3 is significantly decreased in the adipose tissue of CP compared with both Lean and WKY in both 6 and 25 weeks. Conclusion: The proteins associated with adipocyte lipolysis may contribute to the pathogenesis or progression of metabolic syndrome.

PS 1447 DEVELOPMENTAL CONTAMINANT EXPOSURE IMPACTS PHYSIOLOGICAL FUNCTION IN AMERICAN ALLIGATORS, *ALLIGATOR MISSISSIPPIENSIS.*

M. A. Pickett[1], J. T. Martin[1], S. S. Smith[1], E. J. Hulse[1], A. Boggs[2], L. J. Guillette[2, 3] and M. P. Gunderson[1]. [1]*Biology, The College of Idaho, Caldwell, ID,* [2]*Biology, University of Florida, Gainesville, FL and* [3]*Marine Biomedicine and Environmental Sciences Center Medical University South Carolina, Charleston, SC.*
Sponsor: S. Heggland.

Molecular biomarkers provide a useful model for the detection and quantification of physiological alterations elicited by xenobiotics in animal models. However, the extent to which physiology is permanently altered by developmental contaminant exposure and to which it is temporarily altered in response to xenobiotics at later life stages is yet to be determined. In this study we examined three biomarkers, glutathione-S-transferase (GST), lactate dehydrogenase (LDH), and metallothionein (MT) in juvenile alligators from field and developmental studies to determine if early toxin exposure induces physiological alterations. Male and female juvenile alligators were collected from a contaminated site, Apopka and a reference site, Woodruff, and hepatic GST, LDH, and MT were analyzed. We found elevated LDH activity in Apopka males, indicating aerobic stress. GST activity was not significantly different between the two sites. MT levels were significantly higher in Apopka males and slightly elevated in females, indicating heavy metal exposure. In the developmental study, eggs were collected from the same sites, incubated at female producing temperatures, and raised under identical conditions. Hepatic GST, MT and muscular LDH were evaluated to screen for organizational changes elicited by developmental toxin exposure. Significant depression of GST activity in Apopka females suggests that GST is altered by developmental contaminant exposure. This also indicates that GST may be activated to physiologically normal levels in alligators exposed to contaminants through diet and habitat as no difference existed in the field study. Conversely, LDH and MT do not appear to be developmentally altered, but are activated in response to toxins at later life stages. Funding was provided by NIH-INBRE Grant #P20RR016454.

PS 1448 DEVELOPMENT OF A METHOD FOR QUANTITATING SPHINGOID BASE 1-PHOSPHATES IN BLOOD SPOTS.

R. T. Riley[1, 2], N. C. Zitomer[1] and J. Gelineau van Waes[3]. [1]*Toxicology and Mycotoxin Research Unit, USDA-ARS, Athens, GA,* [2]*Interdisciplinary Toxicology Program, University of Georgia, Athens, GA and* [3]*Creighton University School of Medicine, Omaha, NE.*

Red blood cells (RBC) accumulate, store and release sphingoid base 1-phosphates,important ligands for the extracellular receptors $S1P_{1-5}$. The ability of RBC to accumulate these bioactive lipids is because, with the exception of sphingosine kinase, the enzymes responsible for metabolizing sphingosine (So) and sphinganine (Sa) are absent from RBC. In addition, the enzymes responsible for degrading So- and Sa-1-phosphates (So 1-P and Sa 1-P)are lacking in RBC. Thus, sphingoid bases and sphingoid base analogs such as FTY720 are taken up by RBC and are phosphorylated. In mice treated with fumonisin B_1, significant elevation of Sa 1-P in RBC is detected soon after exposure. The half life of phosphorylated sphingoid bases in RBC is short based on the rapid elimination of the phosphorylated sphingoid base analog FTY720. The purpose of this IRB approved study was to develop an analytical method to measure sphingoid bases and sphingoid base 1-P in blood using blood spots collected on FTA® elute micro cards. The FTA® card was chosen because it is impregnated with bactericidal and viricidal chemicals and blood collected on these cards are exempt from shipping restrictions. Blood was collected from finger sticks and allowed to dry at room temperature over night and then stored desiccated at -20°C. An 8 mm core (17.5 µl of blood) was removed from the cards and extracted with acetonitrile/water 5% formic acid. Sa 1-P and So 1-P were easily detected in extracts using liquid chromatography-electro spray ionization-mass spectrometry and levels were proportional to the blood volume of the spot and also the UV (A_{275}) absorbance of the extracts. Sa 1-P and So 1-P were stable for at least 1 month after collection when stored desiccated at -20°C. Extraction efficiencies ranged from 60% to 80% and the limit of detection was less than 2 pmol. The method will be used to study the effects of fumonisin exposure on Sa 1-P levels in human studies in Guatemala.

PS 1449 DEVELOPMENT OF HUMAN EPITHELIAL CELL-BASED ALTERNATIVE MONITORING OF XENOBIOTIC-INDUCED ENDOPLASMIC RETICULUM STRESS.

H. Choi, H. Yang, J. Kim and Y. Moon. *Department of Microbiology and Immunology, Pusan National University School of Medicine and Medical Research Institute, Yangsan, Republic of Korea.*

Based on the ER stress-inducing activity of 8-keto trichothecenes, we developed a bio-monitoring method using intestinal epithelial cells constitutively expressing secretory alkaline phosphatase (SEAP) reporter, which can be used as a sensitive and selective indicator for monitoring ER stress in the epithelial barrier. Type B 8-keto trichothecenes are notable contaminants in cereal-based food stuffs. Epithelial responses are of primary concern during gastrointestinal exposure in human and animal intoxications. Therefore, optimized biomarkers to assess the specific action of trichothecenes on the human epithelial barrier are needed. In the present study, 8-keto trichothecenes were tested to trigger endoplasmic reticulum (ER) stresses that are evident as alteration of eukaryotic initiation factors 2 α phosphorylation and other ER stress markers in human intestinal epithelial cells. Type B 8-keto trichothecenes suppressed the release of SEAP in a dose-dependent manner, showing a negative correlation between logarithm of toxin dose and SEAP activity. In order to correct the monitoring of 8-keto trichothecene in crop matrix, the bioassay was proven to work in standard cereal extract of corn and rice. The mechanism-based monitoring of 8-keto trichothecenes is promising as a supplementary analysis means of the presence of muco-active xenobiotics which are potentially exposed in human gut epithelia (This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2009-0087028)).

PS 1450 INTEGRATION OF TRANSCRIPTOMIC AND PROTEOMIC DATA TO IDENTIFY SUSCEPTIBILITY FACTORS FOR PULMONARY INFECTION DURING DIET-INDUCED OBESITY IN MICE EXPOSED TO LIPOPOLYSACCHARIDE.

S. C. Tilton, K. M. Waters, B. Webb-Robertson, N. J. Karin, X. Zhang, J. M. Jacobs, J. G. Pounds and R. A. Corley. *Pacific Northwest National Laboratory, Richland, WA.*

The systemic effects of obesity on the immune system are poorly understood, but often associated with increased susceptibility to bacterial infections and increased risk for chronic pulmonary disease. Obesity increases sensitivity to endotoxin-induced liver injury, but effects on the lung are uncharacterized. To identify the key biological pathways that define susceptibility factors for pulmonary infection during obesity, we performed parallel exposures of regular weight (RW) and diet-induced obese (DIO) C57BL/6 mice to 0.5ug/L lipopolysaccharide (LPS) by inhalation for 1hr/d for 4 days over a period of 2 weeks. Microarray analysis identified 4893 genes significantly regulated in the study ($p<0.01$) including 2584 genes specific to the LPS response in RW mice and 3475 genes altered by obesity in LPS treated animals. The most significant processes regulated by LPS in mouse lung include those relevant for immune response, leukocyte chemotaxis, cell adhesion, interferon-gamma signaling and cytoskeletal remodeling. Processes were exacerbated in DIO animals along with several unique pathways, such as interleukin signaling, antigen presentation and reactive oxygen production, suggesting that DIO reprograms the lung's transcriptional response to LPS. Global proteomics analysis of lung tissue by LC MS/MS further identified signatures of peptides that describe both obesity and LPS exposure. Approximately 50 percent of these were oxidatively-modified by LPS treatment in RW animals and may serve as useful exposure-specific biomarkers. Integration of gene and protein data revealed a strong overlap of the functions significant for LPS treated animals, including those describing cytoskeletal remodeling and cell adhesion. These results demonstrate biosignatures of systemic inflammation and oxidative stress in obese mice, which may make them more sensitive to environmental lung toxicants. Supported by U54 ES016015.

PS 1451 CERIVASTATIN INDUCES THE MUSCLE ATROPHY PROGRAM IN A TIME- AND FIBER-DEPENDENT MANNER WITH AN INCREASE IN BIOMARKERS OF MYOPATHY.

J. D. Vassallo[1, 2], J. A. Rathmacher[3], E. B. Janovitz[1], S. Stryker[1], D. Robertson[1], L. J. Lowe-Krentz[2] and L. D. Lehman-McKeeman[1]. [1]*Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ,* [2]*Biological Sciences, Lehigh University, Bethlehem, PA and* [3]*Metabolic Technologies, Ames, IA.*

The mechanism of statin-induced myopathy is unknown but recent evidence suggests the ubiquitin ligases MuRF1 and atrogin-1 are involved. Statin myotoxicity occurs in fast-twitch fibers whereas slow-twitch fibers are resistant. It was hypothesized that fiber differences in statin myopathy are dependent on regulation of the ubiquitin ligases. Fast-(psoas) and slow-(soleus) twitch muscles from female SD rats were collected following 1, 6, 8, 10 and 14 doses of cerivastatin (1 mg/kg/day). In the psoas, myopathy was first observed after 8 days, and increased in severity with time. In contrast, no myopathy was observed in the soleus. Transcriptional changes in MuRF1 and atrogin-1 were evaluated by RT-PCR. In the psoas, MuRF1 increased by 2-fold after 6 days whereas atrogin-1 increased by 5-fold after 8 days, and both genes remained elevated on subsequent days. Consistent with induction of the ubiquitin ligases, serum 3-methylhistidine, a marker of myofibrillar protein degradation and necrosis, increased after 8 days, whereas other biomarkers of myotoxicity (skeletal muscle troponin I) were unchanged. In contrast, MuRF1 and atrogin-1 were increased by 3- and 2-fold, respectively in the soleus only after 14 days. The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) inhibits muscle atrophy, a process dependent on induction of MuRF1 and atrogin-1. In the psoas, PGC-1α mRNA was induced by 2-fold on days 6 and 8 but decreased by 2-fold on day 14. In contrast, PGC-1α was unchanged on all days in the soleus however constitutive levels were 4-times higher compared to the psoas. Together, these data demonstrate that MuRF1, atrogin-1 and PGC-1α mRNA are altered by cerivastatin in a time- and fiber-dependent manner. These fiber specific transcriptional changes are consistent with a role for the muscle atrophy program in statin muscle necrosis.

PS 1452 USE OF A QUANTITATIVE IMMUNOHISTOCHEMICAL METHOD FOR DIAGNOSING PHOSPHOLIPIDOSIS.

K. A. Haskins[1], S. Stewart[1], D. Mans[2], J. Zhang[1], D. Peters[1], T. Colatsky[1] and K. L. Thompson[1]. [1]*Division of Applied Pharmacology Research, CDER, U.S. FDA, Silver Spring, MD and* [2]*Division of Pharmaceutical Analysis, CDER, U.S. FDA, St. Louis, MO.*

Phospholipidosis is a lipid storage disorder characterized by phospholipid accumulation in lysosomes that can be induced by more than 250 approved and experimental drugs. While the condition may be considered an adaptive response to drug exposure that protects the cell from xenobiotics, it remains a concern for regulators because of its similarity with lysosomal storage diseases that can cause severe symptoms. The standard method for identifying phospholipidosis is the detection of lamellar bodies using transmission electron microscopy (TEM). This method is labor intensive and low-throughput, making it difficult to incorporate routinely

into preclinical studies. We evaluated a new method for diagnosing phospholipidosis that combines LAMP-2 (lysosomal associated membrane protein-2) immunohistochemical staining with digital image analysis. In cell culture assays, LAMP-2 staining is specific for drug-induced phospholipidosis as detected by quantitation of fluorescent phospholipid accumulation. In Fisher rats treated with coralgil, a drug known to induce generalized phospholipidosis, a significant increase in LAMP-2 staining was measured in tissues with a reported finding of vacuolated macrophages (spleen, mesenteric lymph node, and lung) and in tissues with a reported finding of microvesicular vacuolation (liver), both of which were confirmed as phospholipidosis by TEM. No increase in LAMP-2 staining was seen in skeletal muscle, where stress-induced vacuolation was observed. Because an influx of macrophages - a common pathology of phospholipidosis - can increase LAMP-2 staining, as a control for potential false positive results, immunohistochemistry with CD68 (ED1), a macrophage specific lysosomal protein, was performed to calculate macrophage cell counts. The quantitation of LAMP-2 immunohistochemical staining, in addition to other immunohistochemical approaches, may be an effective tool for diagnosing phospholipidosis in preclinical studies.

PS 1453 BIOCHEMICAL MODULATION OF COPPER NANOPARTICLE UPTAKE DIMINISHES CYTOTOXICITY.

J. B. Lin, A. Schrand and S. M. Hussain. *Applied Biotechnology, AFRL/RHPB, Wright-Patterson AFB, OH.*

Copper particles have been heavily researched for their applications as antimicrobial and catalytic agents. Nonetheless, their toxicity has limited the feasibility of their wide-spread application in living systems. This study demonstrates that dose-dependent cytotoxicity of micro- (~480nm) and nano-sized metallic Cu particles (~80-90nm) in the murine neuroblastoma (N2A) cell line is linked to their endocytic uptake. The addition of a chemical to inhibit endocytosis (Dynasore, 25 μM) resulted in the rapid remediation of cytotoxicity as measured with multiple toxicity endpoints (mitochondrial function, lysosome number). However, it was critical that the cells were pre-treated with the inhibitor for 1 h prior to exposure to a combination of both the inhibitor and copper particles for 3 h. Evaluation of multiple physicochemical parameters and conditions (ie. size in solution, chemical interactions) were used to develop a treatment paradigm leading to significant decreases in toxicity at particle concentrations > 50 μg/mL. Application of this method to other cell types may prove to be a viable method for modulating the uptake of micro- and nano-based particles and their subsequent cytotoxicity.

PS 1454 EFFECT OF ENGINEERED TITANIUM DIOXIDE NANOPARTICLE SHAPE ON TOXICITY *IN VIVO.*

D. W. Porter[1, 2], M. G. Wolfarth[1], N. Wu[3], A. Holian[4], A. Hubbs[1], K. A. Funk[5] and V. Castranova[1, 2]. [1]*HELD/PPRB, NIOSH, Morgantown, WV,* [2]*Physiology and Pharmacology, West Virgina University, Morgantown, WV,* [3]*Mechanical and Aerospace Engineering, West Virgina University, Morgantown, WV,* [4]*Center for Environmental Health Sciences, University of Montana, Missoula, MT and* [5]*Experimental Pathology Laboratories, Inc., Sterling, VA.*

The hypothesis of this study is that nanoparticle (NP) shape will affect pulmonary inflammation and fibrosis. To test this hypothesis, three types of anatase TiO_2 NPs were used: nanospheres (NS), short nanobelts (NB-1) and long nanobelts (NB-2). For in vivo studies, we exposed C57Bl/6 mice by pharyngeal aspiration to TiO_2 nanoparticles (0-30 μg/mouse). Whole lung lavage (WLL) and histopathology studies were conducted. WLL demonstrated that TiO_2 NPs induced dose- and time-dependent pulmonary inflammation (WLL PMNs) and damage (WLL fluid albumin concentration and lactate dehydrogenase activity). Cytokine and chemokine inflammatory mediators also showed dose- and time-dependent changes in WLL fluid. The pattern of responses was NS∠NB-1∠NB-2. Pulmonary clearance of NS and NB-2 indicated that relative to initial deposition, at 28 days post-exposure 69% of NS were cleared verusus 54% for NB-2, and at 112 days post-exposure 96% of NS were cleared versus 80% for NB-2. Histopathology studies showed that at 28 days post-exposure, TiO_2 NPs were observed in alveolar macrophages within the lung, and to a lesser degree within the tracheal-bronchiolar lymph nodes (TBLN). There was an increase in the incidence of the latter finding at 112 days post-exposure, suggesting NP clearance from the lung to the lymph nodes. Persistent alveolitis was observed in mice exposed to NB-1 and NB-2. Interstitial fibrosis was only observed in the NB-1 and NB-2 groups at 28 days post-exposure, and this change persisted at an increased incidence at 112 days post-exposure. Taken together, the data suggest that NP shape does affect lung toxicity and exposure to TiO_2 nanobelts may result in adverse health outcomes. Supported in part by grant RC2 ES-018742 (A. Holian, PI) and NSF grant CBET-0834233 (N. Wu, PI).

PS 1455 INFLUENCE OF THE AGGLOMERATION STATE ON *IN VIVO* PULMONARY INFLAMMATION AND CYTOTOXICITY OF INHALED NANO-AEROSOLS OF TIO2.

A. Noel[1], K. Maghni[2], Y. Cloutier[3], C. Dion[3], R. Tardif[1] and G. Truchon[3]. [1]*Environmental and Occupational Health, University of Montreal, Montreal, QC, Canada,* [2]*University of Montreal, Montreal, QC, Canada and* [3]*Institut de Recherche Robert-Sauvé en Santé et en Sécurité du Travail, Montreal, QC, Canada.*

Nanoparticle (NP) size may be a critical parameter related to toxicity. Few studies have investigated the influence of the agglomeration state on the correlation between the characteristics of the NP exposure dose (including agglomerates size in aerosols) and the toxic effects. The objective of this study was to evaluate in vivo the pulmonary toxicity of nano-TiO2 aerosols of different agglomeration states, strongly (>100 nm) and lightly agglomerated (<100 nm), at equal mass concentrations of 2 and 7 mg/m3. Four groups of Fisher 344 male rats were nose-only exposed to different agglomeration state 5 nm TiO2 aerosols for 6 hr. Two control groups were exposed to clean air. Dry generation methods (fluidized bed, TSI; Palas, GmBH) and a wet method (Collison, BGI) were used to aerosolize the NP powder. The average exposure concentrations in the inhalation chamber measured gravimetrically were 2.024; 1.958; 7.119 and 7.147 mg/m3; respective median number aerodynamic diameters provided by an electrical low pressure impactor (Dekati) were 30; 185; 31 and 194 nm. Rat broncho-alveolar lavage fluids (BALF) collected 16 hr after exposure were analyzed for pulmonary inflammation (total and differential cell count) and cytotoxicity (lactate dehydrogenase, alkaline phosphatase, glutathione, protein concentration). All exposed rats showed an increase in neutrophil count from BALF compared to control. However, a significant 2.1 fold increase ($p<0.05$) was observed only in rats exposed to the 7 mg/m3 strongly agglomerated aerosol, suggesting an inflammatory effect. No other significant differences were noted. Our findings indicate that exposure to 2 mg/m3 TiO2 NP, lightly or strongly agglomerated, do not induce significant pulmonary effects. However, the influence of the agglomeration state on pulmonary toxicity seems to be discriminated at a concentration of 7 mg/m3 with the strongly agglomerated aerosol.

PS 1456 EFFECT OF SILVER NANOPARTICLES ON NITRIC OXIDE RELEASE IN THE RAT TRACHEA SMOOTH MUSCLE.

C. Gonzalez[1], S. Salazar-Garcia[1], P. P. Martinez-Cuevas[1], A. Ramirez-Lee[1], S. E. Jimenez-Badillo[1], H. Rosas-Hernandez[1] and S. F. Ali[2]. [1]*Faculty of Chemistry, Autonomous University of San Luis Potosi, San Luis Potosi, SLP, Mexico and* [2]*Neurochem Lab. ., Division of Neurotoxicology, NCTR/FDA, Jefferson, AR.*

Silver nanoparticles (AgNPs) have been used for the generation of materials with new properties and functions. However, few experimental approaches have been used to assess their potential toxic or beneficial effects on human health. Little is known about their actions related to the nature, size, concentration and biological target. The aim of this work was to evaluate the effects of the AgNPs upon the smooth muscle of trachea in rat, using an isolated system. A single administration of AgNPs, did not modify the muscle tone. However when the trachea rings where pre-treated with acetylcholine (ACh), followed by a single dose of AgNPs, a contractile effect was exerted. Simultaneous administration of AgNPs and ACh showed a slight increase on the contractile effects induced by ACh, however, the AgNPs pretreatment followed an ACh administration, showed that AgNPs exerted an important contraction induced by ACh, and did not return to the basal level. This physiologic effect was associated with an increase in the production of nitric oxide (NO) when compared with control. In order to investigate the possible role of NO on the contractile response of the AgNPs, we incubated the trachea rings in presence of 1400 W (specific blocker of the inducible nitric oxide synthase, enzyme that generate high levels of NO, during inflammation). After the administration of ACh, and before administration of the AgNPs, a contractile response of the AgNPs induced by ACh, was completely blocked. These data suggest that AgNPs modify the contractile action of the ACh, through induction of the NO production. AgNPs may exert hyper reactivity in the air pathway as well.

PS 1457 GLOBAL GENE PROFILING REVEALS DOSE AND TIME PROGRESSION OF KEY BIOLOGICAL RESPONSES OF LUNG EPITHELIAL CELLS TO NANOSCALE AMORPHOUS SILICA *IN VITRO.*

N. J. Karin[1, 3], K. M. Waters[1, 3], J. G. Teeguarden[1, 3], W. Wang[2, 3], J. A. Price[3], R. D. Quesenberry[1, 3] and B. D. Thrall[1, 3]. [1]*Pacific Northwest National Laboratory, Richland, WA,* [2]*Oak Ridge National Laboratory, Oak Ridge, TN and* [3]*Battelle Center for Fundamental and Applied Systems Toxicology, Columbus, OH.*

Nanoscale amorphous silica is used widely in consumer products and is a representative for poorly soluble, low toxicity metal oxide nanoparticles. We used global gene expression profiling to identify key mode-of-action related cellular processes and to quantify impacts on these processes as a function of time and target cell silica particle surface area dose. Mouse C10 lung epithelial cells in submerged culture were exposed to four concentrations of 33-nm carboxylated amorphous silica (10, 50, 100 and 250 μg/ml) for 2, 4, 8 and 24 hours. Cell viability was unaffected by silica at concentrations ≤ 50 μg/ml, while treatment at 100 and 250 μg/ml decreased cell viability by 6% and 17%, respectively. Global transcriptomic profiling (Affymetrix DNA arrays) showed a distinct progression, increasing with dose and time, with unique expression patterns where toxicity was evident and absent. Among the gene products most robustly up-regulated by non-cytotoxic exposures were transcripts linked to inflammation (Ccl7/Cxcl1/Cxcl5/Cxcl15/ Ptgs2), angiogenesis/wound repair (Ankrd1/Ang2/Ang4/Angptl2/figf), oxidative stress (Olr1/Cybb) and lung tissue remodeling/injury response (Mmp3/Mmp8/Mmp10/Mmp13). Most of these changes in transcript level progressed in intensity from low non-cytotoxic exposures to cytotoxic exposures. We calculated the delivered particle surface area for each exposure using a particokinetic model for particles in vitro, which enabled separation of the individual roles of cellular dose and exposure duration. By de-convoluting exposure-duration and dose, the dependence of the response for key biological processes could be quantified and related to comparable acute human exposure levels by the inhalation route.

PS 1458 PULMONARY TOXICITY OF AMORPHOUS SILICA NANOMATERIAL: A COMPARISON OF INHALATION AND INSTILLATION STUDIES.

L. Ma-Hock[1], M. Wiemann[3], S. Brill[1], W. Wohlleben[2, 1], V. Strauss[1], S. Treumann[1], B. van Ravenzwaay[1] and R. Landsiedel[1]. [1]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany,* [2]*ibe, Marl, Germany and* [3]*Polymer Physics, BASF SE, Ludwigshafen am Rhein, Germany.*

The inhalation and deposition of air-borne nanomaterials in the lung is a complex process. Their potential pulmonary toxicity should thus be studied by inhalation exposure. Those studies demand, however, special equipment and large quantities of test material. Intratracheal instillation appears as a simple and less substance-consuming alternative, although bolus dosing and the more central distribution of the particles in the lung are a well known trade-off. We compared the response of the lung to amorphous silica (AS) after instillation and inhalation. For inhalation the established short-term protocol for nanomaterials was employed (Ma-Hock et al. Inhal. Tox. 21:102, 2009): Rats inhaled for 6 h/day on 5 consecutive days 0, 0.5, 2.0 or 10 mg/m3 of either uncoated or polyacrylate coated AS nanomaterial. The bronchoalveolar lavage fluid (BALF) and lungs (histopathology) were examined 3 days and 3 weeks after the end of the exposure. In parallel, rats were intratracheally instilled and equally evaluated. The instilled dose corresponded to the aerosol concentration used for inhalation. After instillation, coated and uncoated AS elicited significant changes in BALF and moderate to massive multifocal granulomatous inflammation in the lungs. In contrast, inhalation of the coated and uncoated silica caused no, or minimal inflammatory effects. Coated AS applied by both exposure techniques caused a significant but reversible increase of spleen weights. An increased spleen weight was also observed after instillation, but not inhalation, of the uncoated AS. Results show that inhalation and instillation of nominally equal mounts of AS elicit different results in the lung with inhalative treatment being less harmfull. This difference may be due to the bolus effect inevitable linked to instillation. Instillation stuldies with AS may, therefore, be of limited value with respect to dose-response assessment.

PS 1459 NANOPARTICLE INHALATION ENHANCES CARDIAC PROTEIN PHOSPHORYLATION AND NEUROTRANSMITTER SYNTHESIS IN THE NODOSE GANGLIA OF RATS.

H. Kan[1], Z. Wu[2], S. Young[1], T. B. Chen[1], J. L. Cumpston[1], F. Chen[1] and V. Castranova[1]. [1]*HELD/PPRB, NIOSH, Morgantown, WV and* [2]*Department of Neurobiology and Anatomy, West Virginia University, Morgantown, WV.*

Growing evidence from epidemiological studies indicates that an increase of the small sized particle, particularly nanoparticle, component in ambient air is strongly associated with increased incidence of cardiovascular diseases. Animal studies demonstrated that pulmonary inhalation of nanoparticles stimulates the development of atherosclerosis and impairs vascular function. However, the effect of inhaled nanoparticles on cardiac muscle has not been reported. The present study investigated the effect of ultrafine titanium dioxide (UFTiO2) on the heart. We found that direct exposure of rat cardiac myocytes in vitro to UFTiO2 (1 μg/ml) for 4 hrs did not alter myocyte contractility, calcium handling, or the phosphorylation level of cardiac proteins, such as p38 mitogen-activated protein kinase (p38 MAPK) and cardiac troponin I (cTnI). In contrast, pulmonary inhalation of UFTiO2 (6 mg/m3) for 4 hrs significantly increased the phosphorylation status of p38 MAPK and cardiac cTnI in the heart. In addition, pulmonary exposure to UFTiO2 also increased neurotransmitter substance P synthesis in nodose ganglia, which is involved in the integration and control of lung and heart function. However, neither mRNA expression nor protein synthesis of TNF-α, IL-1 and IL-6 was detected in the heart or peripheral blood respectively. Blood cell counts and differentials did not indicate significant systemic inflammation. Our results suggested that pulmonary exposure to UFTiO2-enhanced the phosphorylation level of p38 MAPK and cTnI in cardiac tissue. Such responses may contribute to cardiac dysfunction which is independent of the direct interaction of UFTiO2 with the heart or systemic inflammation, but may involve a lung-neuron-regulated pathway.

PS 1460 DISTRIBUTION, ELIMINATION, AND BIOPERSISTENCE TO 90 DAYS OF A SYSTEMICALLY-INTRODUCED 30 NM CERIA ENGINEERED NANOMATERIAL IN RATS.

R. A. Yokel[1], T. C. Au[1], M. Dan[1], R. L. Florence[1], J. M. Unrine[2], R. C. MacPhail[4], M. T. Tseng[3], D. Butterfield[2], S. S. Hardas[2], R. Sultana[2], U. M. Graham[2], P. Wu[2] and E. A. Grulke[2]. [1]*Pharmaceutical Sciences, University of Kentucky, Lexington, KY,* [2]*University of Kentucky, Lexington, KY,* [3]*University of Louisville, Louisville, KY and* [4]*U.S. EPA, Research Triangle Park, NC.*

Background: Nanoscale ceria is a diesel fuel additive, among other applications. Objectives: To extend our prior work showing no reduction of cerium in reticuloendothelial tissues up to 30 days after a single dose of nanoscale ceria, determine the routes and rate of its excretion, and further characterize its distribution, persistence, and effects in the rat. Methods: An ~ 5% aqueous dispersion of citrate-stabilized 30 nm ceria, synthesized and characterized in-house, was iv infused into 16 rats (87 mg/kg), which were terminated 1, 7, 30 or 90 days later. Fifteen control rats received vehicle. Three rats received 50 mg/kg cerium(III) ion. Rats were housed in metabolism cages for up to 2 weeks to quantify urinary and fecal cerium output, cage-side observations recorded daily, and weighed weekly. At termination, nine organs were weighed and samples were collected from 14 organs/systems, blood and CSF for cerium determination by ICP-MS. Results: Nanoscale ceria was less acutely toxic than the cerium ion. Less than 1% of the ceria or cerium ion dose was excreted in the first week; 98% was in feces. Body weight gain in ceria-treated rats was significantly lower than controls. Ceria was primarily retained in the spleen, liver and bone marrow. Spleen weight was significantly increased in some ceria-treated groups, associated with visual evidence of abnormalities. Conclusions: Nanoscale ceria and the cerium ion were retained by reticuloendothelial tissues from which it was very slowly cleared. These results further support the concern about the long term fate and adverse effects of inert nanoscale metal oxides that reach systemic circulation, from which they can distribute throughout the body, resulting in persistent retention and potential adverse effects in multiple organs. Supported by U.S. EPA STAR Grant RD-833772.

PS 1461 PHOTO-ACTIVATION OF MULTISIZED TITANIUM DIOXIDE NANOPARTICLES (TiO_2NPS) RESULTS IN DIFFERENT ROS PRODUCTION AND OXIDATIVE TOXICITY PROFILES IN ZEBRAFISH EMBRYOS.

O. Bar-Ilan[1, 2], K. Louis[2], S. Yang[2], J. Pedersen[2], R. Hamers[2], R. E. Peterson[1, 2] and W. Heideman[1, 2]. [1]*Division of Pharmaceutical Sciences, University of Wisconsin, Madison, WI and* [2]*NSEC, University of Wisconsin, Madison, WI.*

When TiO_2NPs absorb photons of sufficient energy, electrons are excited from valence to conduction band, generating electron-hole pairs. These photo-activated (*p*-) TiO_2NPs can interact with H_2O or O_2, creating reactive oxygen species (ROS). This can overwhelm neutralizing antioxidant cellular defenses, resulting in ROS damage to important cellular macromolecules, leading to death. To investigate the environmental implications of TiO_2NPs photochemistry, we used zebrafish as a model aquatic organism for exposure to two commercial and four lab-synthesized preparations of TiO_2NPs ranging in modal size from approximately 6-30 nm. We hypothesized that photo-activation of TiO_2NPs would induce oxidative toxicity in zebrafish embryos, and that the potency in producing this toxicity would be affected by size and phase type of the particles. Continuous exposure to TiO_2NPs during development resulted in significant mortality and incidence of malformations (stunted growth and craniofacial, yolk, tail, and circulatory malformations), which were dependent on illumination. Differences in toxicity and ROS production were found between commercial and lab-made TiO_2NPs, as well as between otherwise identical preparations of different sizes. A transgenic zebrafish reporter line *Tg(are:eGFP)* was created, coupling a GFP reporter with antioxidant response elements (AREs) to help assess the production of ROS *in vivo*. ARE activation was detected only in embryos exposed to TiO_2NPs and illumination. Assessment of DNA damage by ELISA for 8-OHdG also showed increased levels of oxidized bases in embryos exposed to *p*-TiO_2NPs. Pulse exposures, TEM, and ICP-OES revealed uptake/association of the TiO_2NPs by embryos, with the chorion as a protective barrier. Rescue of the toxic effects by pre-exposure to N-acetylcysteine for oxidative stress protection demonstrated that *p*-TiO_2NPs lead to oxidative toxicity in zebrafish embryos. NSF award DMR-0425880.

PS 1462 PULMONARY TOXICITY OF BIODIESEL PARTICULATE MATTER.

A. A. Shvedova[1], E. Kisin[1], A. R. Murray[1], A. Tkach[1], D. Schwegler-Berry[1], S. H. Young[1], V. E. Kagan[2] and A. D. Bugarski[3]. [1]*PPRB, NIOSH, Morgantown, WV,* [2]*Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and* [3]*OMSHR, NIOSH, Pittsburgh, PA.*

Biodiesel is the only alternative fuel that currently meets requirements of the Clean Air Act. The use of biodiesel results in a substantial reduction of unburned hydrocarbons, carbon monoxide, and particulate matter (PM) as compared to diesel emissions. The broad use of biodiesel in mining and other industries urge assessments of their health effects. We hypothesized that biodiesel PM is toxic to the lung and the toxicity is dependent on at least three major mechanisms: direct reactivity of biodiesel PM electrophiles towards critical biomolecules, induction of robust inflammatory response associated with the nano-sized components of biodiesel PM, and triggering of oxidative stress pathways via depletion of essential antioxidant molecules and activation of oxidative burst in inflammatory cells. In the current study, we compare toxic effects of neat or blended biodiesel PM *in vitro* and *in vivo*. Here, we report that biodiesel PM is cytotoxic to RAW 264.7 macrophages and triggering oxidative stress pathways via depletion of essential antioxidant molecules such as GSH. Our preliminary *in vivo* data indicating that pharyngeal aspiration exposures to biodiesel PM resulted in an augmentation of biomarkers of inflammation and oxidative stress as evidenced by significant depletion of antioxidants in mouse lungs, recruitment of inflammatory cells, changes in permeability of the lung epithelium, pulmonary cell damage and increase in pro-inflammatory cytokines as assessed in the bronchoalveolar lavage (BAL) fluid. Further investigations are warranted to elucidate the toxic properties of biodiesel PM *in vitro* and *in vivo* and the possible mechanisms involved in such toxicity.

PS 1463 ENGINEERED SILICA NANOPARTICLES ACT AS ADJUVANTS TO ENHANCE ALLERGIC AIRWAY DISEASE IN MICE.

J. R. Harkema[1], N. Rowley[1], L. Bramble[1], Q. Zhang[2], D. Jackson-Humbles[1], G. Baker[2], J. Wagner[1] and M. Worden[3]. [1]*Pathobiology, Michigan State University, East Lansing, MI,* [2]*Chemistry, Michigan State University, East Lansing, MI and* [3]*Chemical Engineering, Michigan State University, East Lansing, MI.*

With the increasing production and use of engineered nanoparticles (ENP; < 100 nm mean diameter), there is growing public concern about potential health effects due to occupational/environmental exposure. Nanosized particles found in environmental air pollution have been associated with increased morbidity of asthma. Ambient nanoparticles have been shown to have adjuvant effects that enhance allergic airway disease in mice. Similar studies of the adjuvant potential of selected ENP are lacking. The purpose of the present study was to investigate the adjuvant potential of silica ENP (SiO2; 90 nm) using a murine model of allergic airway disease. Female BALB/c mice were intranasally sensitized with 0.02% ovalbumin (OVA; allergen) or saline (control), and co-sensitized with 0, 10, 100, or 400 μg of SiO2. OVA-sensitized mice were intranasally challenged with 0.5% OVA two weeks after sensitization. Mice were sacrificed 24 hours after the last intranasal challenge and bronchoalveolar lavage fluid (BALF) was collected. Differential cell counts and cytokines in BALF were determined and left lung lobes were processed for histopathology and morphometric analyses. OVA-induced pulmonary inflammation was enhanced by SiO2 co-sensitization. SiO2 caused a dose-dependent increase of BALF eosinophils in OVA mice (544%, 1,341%, and 2,448% for 10, 100, and 400 μg SiO2 respectively). Similar SiO2 enhancement of OVA-induced neutrophils in BALF was also induced (72%, 107% and 145%). This pattern of dose response was also reflected in the amounts of inflammatory cytokines (e.g., IL-5, IL-6, IL-13, INFγ, KC) in BALF. Intranasal aspiration of SiO2, concurrent with OVA sensitization, caused an adjuvant-like enhancement of OVA-induced allergic airway disease in mice. These results suggest that asthmatics may be particularly susceptible to the adverse health effects caused by airborne exposure to silica ENP. Funded by NIH 5RC2ES018756-02.

PS 1464 COPPER NANOPARTICLES INDUCE CEREBRAL MICROVASCULATURE PROLIFERATION OR TOXICITY THAT AFFECT BLOOD-BRAIN BARRIER PERMEABILITY.

W. J. Trickler[1], S. M. Lantz[1], B. L. Robinson[1], G. D. Newport[1], P. C. Howard[2], J. J. Schlager[3], M. G. Paule[1], W. Slikker[1], S. M. Hussain[3] and S. F. Ali[1]. [1]*Neurochemistry Laborator, Division of Neurotoxicology, NCTR/U.S. FDA, Jefferson, AR,* [2]*Office of Scientific Coordination, NCTR/U.S. FDA, Jefferson, AR and* [3]*AB Branch, AFRL, Wright-Patterson Air Force Base, Dayton, OH.*

The purpose of the current studies was to determine if systemic exposure to copper nanoparticles (CuNPs) can induce the release of pro-inflammatory mediators that influence the restrictive permeability characteristics of the BBB. Our in vitro BBB model, a primary culture of brain microvessel endothelial cells (BMEC),was isolated from fresh porcine or rat cerebral cortices.Confluent rat and porcine BMEC monolayers (10-14 days) were treated with 40 and 60 nm CuNPs. Cytotoxicity was evaluated using a cell proliferation assay (XTT) in BMEC following CuNPs exposure (1.5-to-50 μg/ml, 24 hr). The extracellular concentrations of pro-inflammatory mediators (IL-1β,IL-2, TNFα and PGE2) were evaluated using an ELISA at 0, 2, 4, 6 and 8 hr following CuNPs exposure (25 μg/ml). BMEC cell morphology was monitored microscopically following CuNPs exposure (5 or 25 μg/ml). BMECs were cultured in standard 12-well transwell inserts and permeability was evaluated following 24hr CuNP exposure (25μg/ml) by measuring fluorescein transport and the apparent permeability. CuNPs induced cytotoxicity at concentrations ≥ 12 μg/ml, while lower concentrations induced cell proliferation in BMECs. PGE2 release following CuNP exposure was significantly increased (≈7fold and 3fold at 8 hr after 40 and 60 nm, respectively). Qualitatively similar results were observed for both TNFα (3 fold after 4hrs) and IL-1β (10 fold after 8hrs). The changes in pro-inflammatory events correlated well with increased fluorescein permeability where permeability apparent ratios (exposure/control) were 8 fold for 40 and 60 nm CuNPs. These data suggest that interactions of CuNPs with the BBB may produce pro-inflammatory mediators that could influence BMEC proliferation at low concentrations or induce BBB toxicity and potential neurotoxicity at higher concentrations. Supported by WPAFB/NCTR IAG.

PS 1465 *IN VITRO* TOXICITY OF AMPHIPHILIC POLYMER-COATED CD/SE/ZNS QUANTUM DOTS IN MULTIPLE HUMAN AND MOUSE CELL TYPES.

L. McConnachie, C. White, D. Botta, M. Zadworny, D. Cox, R. Beyer, X. Hu, D. Eaton, X. Gao and T. Kavanagh. *University of Washington, Seattle, WA.*

Recent developments in nanotechnology have produced an array of engineered nanoparticles that possess many unique and interesting properties. However, concerns have been raised regarding their potential to exert deleterious effects on biologic systems. Therefore, it is critical to understand and characterize their potential toxicity. Quantum dots (QDs) are nanoscale (2-10 nm) fluorescent crystals with unique photochemical and photophysical properties. We have synthesized CdSe/ZnS core/shell QDs with a tri-n-octylphospine oxide, poly(maleic anhydride-alt-1-tetradecene) (TOPO-PMAT) coating. We investigated their uptake and toxicity in 5 different cell lines derived from both murine and human origins, in-

cluding mouse and human macrophage, mouse endothelial, human lung epithelial, and human kidney proximal tubule epithelial cell lines. Cells were treated with increasing concentrations of TOPO-PMAT QDs (0-40 nM) for 24 hours and then assessed for QD uptake, cytotoxicity and stress response indicators. We found that both human and murine macrophage cell lines readily internalized these QDs, as did human kidney proximal tubule cells. This relatively high uptake was accompanied by modest toxicity. Mouse endothelial cells and human lung epithelial cells exhibited minimal TOPO-PMAT QD uptake and a low degree of cytotoxicity. Unsupervised hierarchical clustering analysis revealed that TOPO-PMAT QD uptake was highly correlated with the expression of protein-based redox sensors as indicated by the induction of heme oxygenase-1 and glutamate-cysteine ligase modifier subunit, and with NADPH content. We conclude that TOPO-PMAT QD uptake is highly variable among cell types and that these QDs are minimally acutely toxic resulting in mild oxidative stress. Therefore, the effects of quantum dots in vivo will likely be highly dependent upon the cell type and organs being exposed. Supported by NIH grants R01ES016189, P30ES07033 and T32ES07032.

PS 1466 DEVELOPMENT OF NANO-AEROSOL GENERATION TEST PLATFORM FOR INHALATION TOXICOLOGY STUDIES.

V. B. Mikheev[1, 4], W. S. Forsythe[1, 4], B. N. Swita[1, 4], K. R. Minard[2, 4] and W. Wei[3, 4]. [1]*Battelle Toxicology Northwest, Richland, WA,* [2]*Pacific Northwest National Laboratory, Richland, WA,* [3]*Oak Ridge National Laboratory, Oak Ridge, TN and* [4]*B-FAST, Battelle Center for Fundamental and Applied Systems Toxicology, Columbus, OH.* Sponsor: R. Meng.

Increase of engineered nano-material production demands development of methods to assess toxicological effects of nano-particles considering inhalation is a high risk route of exposure. Inhalation toxicity studies with nano-particles require stable generation of nano-sized aerosols followed by appropriate methods of post-exposure analysis. Aerosol generation of nano-particles suspended in water was achieved via Collison type nebulizer followed by drying-dilution with HEPA filtered air. SiO2 particles of 15, 30, 50, and 100 nm size, as well as Fe3O4 particles of 15 nm size with different surface modifications [amine or carboxyl groups] were used. Particle size and concentration were monitored on-line using near real-time aerosol measurement technology, Scanning Mobility Particle Sizer. In addition Electric Low Pressure Impactor measurements were conducted on selected samples and Micro-Orifice Uniform-Deposit Impactor data were also obtained. Gravimetric filter measurements and scanning electron microscopy analyses was performed. Experimental conditions were tuned to obtain controllable and reproducible aerosols of nano-size range that would be appropriate for use in inhalation studies. (Note: aerosol diameter can be adjusted from ~30 to ~100 nm, at particle concentrations up to ~ 10^6 particles per cm^3). For in vivio studies, a nano-aerosol generation test platform was interfaced with a stainless-steel nose only exposure unit (multiple-tier carousel) that allows conduct of multiple simultaneous in-vivo animal exposures. Tests were performed up to 4 hours with continuous aerosol exposure. Preliminary inhalation tests were performed in which mice were exposed to super-paramagnetic iron-oxide (SPIO) nano-particles followed by magnetic particle dosimetry (MPD) analysis. Tissues, urine, and feces were analyzed using MPD to give a detailed picture of particulate deposition & clearance.

PS 1467 TOXICITY STUDY OF SILVER NANOPARTICLES IN *DROSOPHILA MELANOGASTER* USING STRESS SENSITIVE MUTANTS AND GAL4 ENHANCER TRAP SCREENING.

H. Tian, H. Eom, G. Lee, Y. Chung and J. Choi. *University of Seoul, Seoul, Republic of Korea.* Sponsor: D. Ryu.

In this study, to investigate the effect of silver nanoparticles (AgNPs) in *Drosophila melanogaster*, several stress sensitive mutants and GAL4 enhancer trap screenings were applied in a toxicological context. To compare the toxicity of AgNPs with that of Ag ions, toxicity of Ag ion was also investigated using the same endpoints used for AgNPs toxicity assay. The nine potentially stress sensitive mutant strains were selected for toxicity test and the sensitivity of *D.emlanogaster* to AgNPs was screened on these mutant strains as well as, on wildtype, using survival and development indicators, such as, pupation and adult emergence. *Dsor1, p38, hsp83* and *p53* mutant strains were found to be more sensitive to AgNPs exposure than wildtype. To gain a mechanistic understanding of the different sensitivities of the mutant strains, gene expression analyses were also performed on the four genes. Subsequently to isolate novel biomarker for AgNPs, we generated GAL4 enhancer trap lines, and screened for AgNPs-responsive lines by using *lacZ* reporter gene expression profile. Over 300 lines were screened, a GAL4 line, M95, showed significant elevation of GAL4 activity in response to AgNPs treatment. Detailed results will be presented in the conferences.
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PS 1468 SHORT-TERM RAT INHALATION STUDY WITH AEROSOLS GENERATED FROM ACRYLIC ESTER POLYMERS WITH A PROPORTION OF NANOPARTICLES.

K. Wiench[1], L. Ma-Hock[2], V. Strauss[2], S. Gertrud[3], B. van Ravenzwaay[2] and R. Landsiedel[2]. [1]*Product Safety, BASF SE, Ludwigshafen, Germany,* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany and* [3]*Dispersions for Adhesives & Construction Europe, BASF SE, Ludwigshafen, Germany.*

Polymer dispersions are used as binders in many waterborne applications, e.g. adhesives, carpets, non-wovens, paints, paper and paperboard coating, plasters, textile finishing agents. Depending on the intended use of the polymer dispersion, the particle size can widely vary between < 100 nm (0,1 µm) and 10 µm,. Small particle sizes may pose increased pulmonary hazards upon potential inhalation exposure. Therefore three acrylic ester polymer dispersions, (two liquid dispersions and one dispersion powder) with different proportions of nano-sized particles were tested regarding inhalation toxicity.are In a 5-day screening study, male rats were head-only exposed to aerosols generated from the dispersions of acrylic ester polymers with differing nano-sized particle proportions at particle concentrations of 3 and 10 mg/m3, the high concentration exceeding current nuisance dust threshold limit values by a factor of about 3. All materials were intensively characterized before inhalation (i.e. composition, particle size distribution, TEM) as well as the generated inhalation atmosphere, including TEM of the dust particles. Immediately and 19 days after the end of inhalation, necropsies were conducted with major emphasis on respiratory tract histopathology. Three and 23 days after the end of inhalation, bronchoalveolar lavage was performed to screen for pulmonary injury and inflammation. In contrast to the adverse effects known for other materials in short-term inhalation studies, neither preparation of acrylic ester polymers elicited any adverse effect at the end of the inhalation or post-inhalation periods. No shift in toxicity could be observed by the increased proportion of nano-sized polymer particles in the raw material. Under the conditions of this study, the no-observed-adverse-effect levels for those three preparations were >10 mg/m3.

PS 1469 GENOTOXIC EFFECTS OF NANOMATERIALS IN LUNG TISSUE *IN VITRO* AND *IN VIVO*.

M. Schulz[2], K. Wiench[1], K. Chodakowski[2], A. Hartwig[3], S. Brill[2], L. Ma-Hock[2], B. van Ravenzwaay[2] and R. Landsiedel[2]. [1]*Product Safety, BASF SE, Ludwigshafen, Germany,* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany and* [3]*Food Chemistry and Toxicology, Technical University Berlin, Berlin, Germany.*

Nanotechnology offers extraordinary opportunities in various technological fields since the nano-structure can change the properties of materials. This is also why nanomaterials may interact differently with biological systems. For genotoxic effects, the established testing methods were developed for molecules. It is, however, relatively difficult to adequately apply nanomaterials to genotoxicity test systems, since it is the nature of these materials to change their surface properties and agglomeration state in different environments. In vitro methods for genotoxicity testing require the dispersion of the nanomaterial in the culture media. The use of efficient, reproducible and well-documented dispersion protocols is crucial. The particle size distribution and surface coating of the nanomaterials in vitro may, however, be much different from the one found in the body. Moreover, several interferences of nanomaterials with in vitro test systems have been described. Thus the selection of appropriate genotoxicity tests for a nanomaterial in vitro requires information on its material properties and potential fate in the body . We have assessed the genotoxic potential of two amorphous silica nanoparticles of different sizes, by use of Single Cell Gel Electrophoresis (Comet Assay) in a permanent cell line (V79 cells), in Precision Cut Lung Slices (PCLS) from Wistar rats. In addition to in vitro methods, in vivo methods include uptake, distribution, excretion and modification processes and allow the observation of concurrent general toxicity. Both amorphous silica nanoparticles were instilled into the lungs of Wistar rats and

genotoxic effects in the lung (Comet Assay) and bone marrow (Micronucleus Test) were examined along with the general toxicity (e.g. inflammation of the lung). The comparison of the in vitro and in vivo methods revealed that some genotoxic effects observed in vitro could not be observed in the in vivo studies.

PS 1470 COMPARATIVE GENE EXPRESSION PROFILE IN TISSUES OF RATS INTRATRACHEALLY EXPOSED TO CADMIUM-DOPED NANOPARTICLES.

T. Coccini[1], E. Roda[2], M. Fabbri[3], M. Sacco[3], L. Manzo[1, 2] and L. Gribaldo[3]. *[1]Toxicology Division, Maugeri Foundation IRCCS, Institute of Pavia, Pavia, Italy, [2]European Centre for Nanomedicine, University of Pavia, Pavia, Italy and [3]IHCP, European Commission, Joint Research Centre, Ispra, Italy.*

The development of novel nanopharmaceuticals have potential for revolutionizing medical treatment. Engineered cadmium-containing silica nanoparticles (CdNPs) show great promise for targeted drug delivery. However, risk associated with new nanotechnology needs to be assessed, further Cd accumulates in tissues, particularly in kidney, the main target organ, while, when inhaled, it causes several lung pathologies. This in vivo study investigated pulmonary and renal molecular effects of NPCd (1mg/rat) versus CdCl2 (400µg/rat) 7 and 30 days after intratracheal administration. Cd-containing Silica NPs were prepared from commercial nano-size silica powder (20nm average pore size and 240m2/g surface area). Toxicogenomic analysis was performed by the DNA microarray technology to evaluate changes in gene expression of the entire genome. In lung, the total number of modulated genes was: (i) higher after 30 days compared to 7 days post administration for both CdCl2 (229 and 83 genes modulated at 30 and 7 days, respectively) and NPCd (318 genes at 30 days and 180 genes at 7 days, (ii) higher after NPCd vs CdCl2 at both time points considered (318 vs 229 genes at 30 days; and 180 vs 83 genes at 7 days). Most of the genes modulated by NPCd were different than those modulated by CdCl2: at 30 days, NPCd altered several chemokines, calmodulin-like3, cathepsin, whereas CdCl2 prevalently induced changes in albumin, cytochrome P450, coagulation factor. In kidney, the modulating gene effects were already pronounced at the early time point for both Cd and NPCd (373 vs 268 genes, respectively) with a lasting marked effect until 30 days for NPCd compared to Cd (268 vs 140 genes, respectively).
These outcomes support that whole genome analysis represents a valuable approach to assess the whole spectrum of biological responses to Cd-containing nanomaterials (Supp. Italian Ministries of Health, Research, Education-PRIN2007).

PS 1471 NANOPARTICLES ALTER CYCLOOXYGENASE ACTIVITY IN MICROVASCULAR DYSFUNCTION.

T. L. Knuckles[1], J. Yi[1], D. Frazer[2], J. Cumpston[2], B. Chen[2], V. Castranova[2] and T. R. Nurkiewicz[1]. *[1]Physiology and Pharmacology, West Virginia University, Morgantown, WV and [2]National Institute of Occupational Safety and Health, Morgantown, WV.*

The widespread increase in the production and use of nanomaterials has increased the potential for nanoparticle exposure; however, little is known about the biologic effects of nanoparticle inhalation. We have shown that blunted nitric oxide (NO) production significantly contributes to nanoparticle induced microvascular dysfunction. However, it cannot account for all of the microvascular effects of nanoparticle exposure. The purpose of this study was to evaluate alternative mechanisms of microvascular dysfunction. Rats were exposed to nano-TiO_2 (primary particle size ~21 nm) aerosols in our Inhalation Facility at a concentration of 4-5 mg/m^3 with a count median aerodynamic diameter of ~145 nm for 4-6 hours yielding a total calculated lung burden of 30 µg. 24 h post-exposure, the spinotrapezius muscle was prepared for intravital microscopy. We assessed arteriolar function via perivascular nerve stimulation and active hyperemia with or without inhibitors for endogenous NO production or cyclooxygenase (COX) products. NO inhibition increased vasoconstriction and decreased vasodilation in control but not in exposed animals. COX inhibition had no effect on vasoconstriction in either group but overtly blunted vasodilation in exposed animals. Based on these results, we then determined if exposure altered sensitivity to COX products. Suffusion of the muscle with U46619 (1-100 nM), a thromboxane A_2 mimetic, led to a significantly greater vasoconstriction in exposed animals compared to control (max % of control -22 ± 3.4% sham, -31 ± 4.0% exposed). Furthermore, suffusion of iloprost (2.8-28 nM), a prostacyclin mimetic, led to a slight increase in vasodilation in the exposed animals compared to control (max % of control 177 ± 25% sham, 214 ± 41% exposed). Hence, the compensatory mechanism for the NO deficit appears to be COX mediated, through enhanced sensitivity and possibly increased endogenous production. Support: NIH RO1-ES015022, RC1-ES018274 (TRN).

PS 1472 HUMAN CLINICAL STUDIES OF DIESEL EXHAUST PARTICULATE AND IMPLICATION FOR NANOPARTICLE EXPOSURES.

P. A. Valberg[2], T. W. Hesterberg[1], C. M. Long[2], C. A. Lapin[3] and A. K. Hamade[2]. *[1]Navistar, Inc., Chicago, IL, [2]Gradient, Inc., Cambridge, MA and [3]Lapin and Associates, Glendale, CA.*

While there is a lack of health effects data for humans exposed to engineered nanoparticles (ENPs), there are human data for another source of nanoparticle (NP) exposure, notably for the NPs contained in diesel exhaust particulate (DEP). Studies of human volunteers exposed to diesel exhaust (DE) in research settings have been conducted at DEP-NP number concentrations (i.e., greater than 10^6 particles/cm^3) that exceed number concentrations reported for worst-case exposure conditions for workers manufacturing and handling ENPs. These human DE exposure studies, using sensitive physiological instrumentation and well-characterized exposure concentrations and durations, suggest that elevated DE exposures from pre-2007 engines may trigger short-term changes in, for example, lung and systemic inflammation, thrombogenesis, vascular function, and brain activity. Considerable uncertainty remains both as to which DE constituents produce the observed responses (i.e., DEP NPs, DEP mass, DE gases), and as to the implications of the observed short-term changes for the development of disease. Even so, these DE human clinical data do not give evidence of a unique toxicity for NPs as compared to other small particles. Of course, physicochemical properties of toxicological relevance may differ between DEP NPs and other NPs, yet overall, the DE human clinical data do not support the idea that elevated levels of NPs per se (at least in the DEP context) must be acutely toxic by virtue of their nano-sized nature alone.

PS 1473 PULMONARY AND HEMATOLOGICAL EFFECTS IN RATS FOLLOWING A SINGLE INHALATION EXPOSURE TO CEO2 NANOPARTICLES.

K. Dreher, A. Ledbetter, R. Jaskot, M. Odegaard, R. Snyder, J. Richards and N. Coates. *ORD, NHEERL, U.S. EPA, Research Triangle Park, NC.*

Engineered nanomaterials have unknown environmental and health implications due to their novel properties and/or by-products associated with their applications. Combustion studies have shown nanoCe-enabled fuel additives alter the physicochemical properties of diesel emissions (DE) resulting in increased levels of nanoscale particles containing aggregates of CeO2 NPs. The health effects associated with exposure to CeO2 NPs, or DE produced using nanoCeO2 additives, are unknown. To examine the health effects of CeO2 NP exposure, a low consumption generating system was developed capable of producing CeO2 NPs displaying similar 1° size and aggregate size ranges observed in DE produced with a nanoCeO2 additive. Rats were exposed to a single 6h inhalation to either air or CeO2 NPs with a primary diameter of 4 – 6 nm in the 14 – 300 nm size range having a CMD 47 – 69 nm, GSD 1.89 – 2.0 at either: 0.25, 0.5, or 1.9 mg/m^3. Pulmonary toxicity/physiology and hematological effects were examined in control and exposed rats at various post-exposure time points up to 1 month. Pulmonary edema, cytotoxicity and inflammation were evaluated by alterations in bronchoalveolar lavage fluid (BALF): protein, albumin, LDH and cellular differential levels, respectively, while hematological effects were assessed by alterations in blood: WBC, RBC, lymphocyte and hematocrit. Exposure to 1.8 mg/m^3 CeO2 NPs produced no alterations in pulmonary physiology. Significant increase in all BALF endpoints was detected as early as 12 – 24hr post-exposure and remained elevated over control levels at 1 mon post-exposure. Significant decrease in WBC and lymphocytes was observed only at 24h post-exposure. Similar pulmonary effects were observed in rats exposed to 0.5 mg/m^3 CeO2 NPs while no pulmonary effects were seen in rats exposed to 0.25 mg/m^3 CeO2 NPs. The data provide the first evidence that a single acute exposure to low concentrations of CeO2 NPs can induce a transient hematological effect and persistent lung injury. This abstract does not reflect EPA policy.

PS 1474 EFFECTS OF COPPER NANOPARTICLE EXPOSURE ON HOST DEFENSE IN MURINE PULMONARY INFECTION MODEL OF *KLEBSIELLA PNEUMONIAE.*

J. Kim[1], A. Adamcakova-Dodd[2], P. T. O'Shaughnessy[2], V. H. Grassian[1] and P. S. Thorne[1, 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.*

Human exposure to nanoparticles (NPs) and environmental bacteria can occur simultaneously. NPs induce inflammatory responses and oxidative stress but may also have immune-suppressive effects, impairing macrophage function and altering epithelial barrier functions. The purpose of this study was to assess the potential effects associated with the exposure of commercial copper (Cu) NPs using a murine

model of lung inflammation and host defense. We established a murine pulmonary infection model of *Klebsiella pneumoniae* (*K.p.*) to determine if lung bacterial clearance is enhanced or impaired by Cu NP exposure using two different exposure routes, sub-acute inhalation (4 h/d, 10 d) and intratracheal instillation (3, 35, and 100 μg/mouse). Pulmonary response was evaluated by cell enumeration, lung histopathology, total protein, lactate dehydrogenase (LDH) activity, and inflammatory cytokines in bronchoalveolar lavage (BAL) fluid. Cu-exposed mice induced inflammatory responses with increased recruitment of total cells and neutrophils to the lungs as well as increased total protein and LDH activity in BAL fluid. Inflammatory cytokines in BAL fluid increased dramatically including: IL-6, IL-12, GM-CSF, KC, MCP-1, MIP-1α and TNF-α. Both inhalation and instillation exposure to Cu NPs significantly decreased the pulmonary bacterial clearance of *K.p.*-exposed mice measured 24 h after bacterial infection (10^5 CFUs/mouse) following Cu NP exposure versus sham-exposed mice also challenged with *K.p.* We observed Cu NP exposure impaired host defense against bacterial lung infections and a dose-dependent effect of instilled Cu NPs on bacterial clearance in which even our lowest dose demonstrated significantly lower clearance than observed in sham-exposed mice. Thus, Cu NP exposure may lead to increased risk of pulmonary infection due to impaired host defense against bacteria.

PS 1475 MAGNETIC PARTICLE DETECTION (MPD) FOR RAPID CELL & TISSUE DOSIMETRY.

K. R. Minard[1, 4], B. D. Thrall[1, 4], J. G. Teeguarden[1, 4], M. H. Littke[1, 4], V. B. Mikheev[2, 4] and W. Wang[3, 4]. [1]*Pacific Northwest National Laboratory, Richland, WA,* [2]*Battelle, Richland, WA,* [3]*Oak Ridge National Lab., Oak Ridge, TN and* [4]*B-FAST, Battelle Center for Fundamental and Applied Systems Toxicology, Columbus, OH.*

Magnetic nanoparticles are of widespread interest for disease diagnosis, therapy, monitoring treatment, and understanding how different material properties affect biodistribution and toxicity. Nevertheless, accurate measurements of particle amounts in cells, blood and tissue remain a major challenge. To provide accurate dosimetry for emerging applications, we have developed and tested a new detection platform for quickly and easily determining the mass of superparamagnetic nanoparticles in small sample volumes (~250 ul). Measurements use an oscillating magnetic field (100 Gauss/250 kHz), require no sample preparation, are inexpensive to implement, and takes less than a second. Instrument calibrations performed using aqueous suspensions of 15 nm iron oxide (Fe_3O_4) cores with different surface coatings (plain, carboxyl (-COOH), or amine (-$N(CH_3)_3$) exhibit excellent correlation (R^2~0.99) between detector output and total particle mass. Data further demonstrates that detection limits are typically less than ~100 ng, and that sensor output is insensitive to particle agglomeration or testing matrix (cell, media, or tissue). Results from *in vitro* studies show that the uptake of carboxyl-coated iron oxides in RAW 264.7 macrophages is nearly six-fold lower in knockouts with a 90% lower expression of the scavenger receptor, and that uptake is well described by a particokinetic model that accounts for experimental conditions. To demonstrate *ex vivo* dosimetry, excised mouse tissues were analyzed after a 4 hr exposure to aerosolized Fe_3O_4-COOH having a count median aerodynamic diameter of ~70 nm & a concentration of ~1900 ug/m^3. Results highlight rapid lung clearance over a 7-day period, and in combination with other findings, demonstrate MPD's broad utility for important dosimetry applications. *Funded by NIBIB R21 EB008192, NHLBI R01 HL073598, NIEHS R01 ES016212 and Battelle Memorial Institute.*

PS 1476 SYNTHESIS, CHARACTERIZATION, AND TOXICITY STUDIES OF DISCRETE SIZE NANOPARTICLE-PESTICIDE CONJUGATE FOR NOVEL PESTICIDE DELIVERY.

A. Sooresh, H. Kwon, P. Pietrantonio, M. Pine and C. Sayes. *Texas A&M University, College Station, TX.*

Extensive research has been done on the synthesis, characterization and toxicity studies of silver nanoparticles. The current literature suggests that silver in a nanoparticulate form induces little adverse effects; therefore nanosilver has the potential to be utilized in a variety of environmental applications. This work proposes a novel synthetic scheme to produce a nanoparticle-pesticide conjugate to be used as an active agent against insect vectors such as mosquitoes. Monodispersed silver nanoparticles were synthesized by chemically reducing a silver salt and then conjugated to deltamethrin, a synthetic pyrethroid pesticide, resulting in a stable colloidal suspension. This pesticide encapsulated nanoparticle (PEN) was characterized using a variety of techniques including UV-Visible Spectroscopy, Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Spectroscopy (EDS) and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). The newly developed PEN was tested on mosquitoes to assess their mortality and behavior. Data from the mosquito bioassay confirms that PEN is lethal to mosquitoes after a 24 hour exposure. These results demonstrate the potential to use nanoparticle surfaces in disease prevention. In particular, the nanoparticle-pesticide conjugates will find wide applications as they can be impregnated into bed nets, clothing and household fabrics, as well as paints and other coatings, to help prevent disease and potentially decrease human exposure to toxic chemicals. Currently, cultured cell lines are being utilized to assess the cytotoxicity of PEN on mammalian systems.

PS 1477 NANOPARTICLE TOXICITY ON AIRWAY EPITHELIAL CELLS.

M. M. McCorkel[1, 2] and S. Boitano[1, 2, 3]. [1]*Biomedical Engineering, University of Arizona, Tucson, AZ,* [2]*Arizona Respiratory Center, Arizona Health Sciences Center, Tucson, AZ and* [3]*Physiology, University of Arizona, Tucson, AZ.* Sponsor: R. Lantz.

There are strong correlations between ultrafine particle deposition in the lung and chronic respiratory illness. The emergence of engineered nanoparticles (ENPs) and their growing prevalence in society presents a new potential lung toxicant exposure with limited known effects on lung health. Recent research on chronic obstructive pulmonary disease associated with ultrafine particle deposition has uncovered a key role for dysregulation of paracrine ATP signaling and corresponding innate immune function. We hypothesized that ENP exposure on lung cells may affect ATP response in the lung epithelium and this would limit cell adhesion and migration during wound repair. We examined ATP signaling and compromise of innate immune function in cultured human airway epithelial cells (16HBE14o-) following exposure to nano-sized (20 nm) hafnium oxide (HfO_2) as a model ENP. After incubation with low doses of HfO_2 (50 and 250 mg/L), cells were examined with high-throughput real time cell analysis (RTCA) assays and digital imaging video microscopy to evaluate changes in ATP-induced cell signaling, cell adhesion, cell migration and wound repair. Lung epithelial cells displayed an altered response to ATP, reduced cell adhesion, cell migration and wound repair following 24 hr exposure to HfO_2. These results suggest that low doses of HfO_2 alter a basic innate immune function in lung epithelial cells that could contribute to respiratory disease.

PS 1478 TRANSPORT MECHANISM OF NANOPARTICLE INTO THE BRAIN.

B. Choi, D. Kim and J. Park. *Preventive Medicine, Chung-Ang University, Seoul, Republic of Korea.*

Nanomaterials can be used in various biomedical and cosmetic applications, however, the neurotoxicity of these materials was poorly understood. Purpose of the present study was to establish the in vitro blood-brain barrier(BBB), blood-CSF barrier(BCB) models and in situ brain perfusion technique to screen the transport of nanomaterials into the brain. We also performed a transport study using in vitro with silver nanoparticle (AgNP) and different size gold nanoparticles (AuNPs) and did a transport study using in situ method with AgNP. We established the in vitro BBB and BCB models which was used co-culture method with ECV304 cell and C6 glioma cell and was used with Z310 cell, respectively. Silver (Ag) and gold (Au) ion uptake was higher than AgNP and AuNPs in BBB and BCB models. In BBB model, the permeability of AuNPs was found to be depending on particule size. Ag uptake was higher than AgNP in situ brain perfusion and Ag and AgNP transport into the brain were mainly achieved through the BBB. These results were similar in vitro study results. Taken together, these data suggest that in vitro BBB model can be used to a screening test for nanomaterials transport into the brain and the smaller sized AuNPs can be transported into the brain easily.

PS 1479 EMBRYONIC GENE EXPRESSION IS IMPACTED BY SURFACE FUNCTIONALITIES OF GOLD NANOPARTICLES.

L. Truong[1, 2], T. Zaikova[2, 3], J. Miller[2, 3], J. Hutchison[2, 3] and R. Tanguay[1, 3]. [1]*Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR,* [2]*The Oregon Nanoscience and Microtechnologies Institute and the Safer Nanomaterials and Nanomanufacturing Initiative, Eugene, OR and* [3]*Chemistry, University of Oregon, Eugene, OR.*

Gold nanoparticles (AuNPs) that were cationic-functionalized, N,N,N trimethylammoniumethanethiol (TMAT), or anionic- functionalized, 2-mercaptoethanesulfonate (MES), with identical 1.5nm core sizes produced differential developmental responses in a sensitive embryonic zebrafish assay. To investigate the molecular mechanisms underlying the differential toxic responses, global gene expression studies were conducted using NimbleGen zebrafish microarrays. RNA was isolated from embryonic zebrafish exposed to the 100% effective concentrations (EC100s) for 1.5nm TMAT-AuNPs (50 ppm) and MES-AuNPs (10 ppm) at 24

and 48 hpf. While the cores of these AuNPS are identical, the presence of the different surface functionality produced unique gene expression changes. At 24 hpf, both MES- and TMAT-AuNP impacted approximately the same number of transcripts. However, at 48 hpf, the number of misregulated transcripts impacted by MES-AuNP increased by ~14 fold, and only ~3 fold for TMAT-AuNP. When surface functionalization effects were assessed, both MES- and TMAT-AuNP impacted 3 genes at both time points. MES-AuNP had nearly twice as many misregulated transcripts as TMAT-AuNP at 48hpf. Preliminary pathway analysis using Ingenuity Pathway Analysis (IPA) identified the most significant biofunction pathways impacted by MES-AuNP were cellular and tissue development related transcripts. For the TMAT-AuNP exposed embryos, gene expression, cell cycle, and proliferation pathways were the most significantly impacted. Collectively, this data indicates that the NP surface functionalization can drive unique biological responses. A more thorough investigation of the role of specific differentially regulated transcripts in producing biological responses is underway. This research is supported by NIEHS 1 F31 ES019445-01, NIEHS P3000210, ES016896, EPA RD-833320, and Air Force Research Laboratory #FA8650-05-1-5041.

PS 1480 DISPERSIBILITY CONTROL AFFECTS THE RESULT OF CYTOTOXICITY TEST FOR NANOMATERIAL.

H. Yang[1], K. Lee[1], C. Lee[2], K. Cho[1], C. Kim[1] and C. Song[1]. [1]*Korea Institute of Toxicology, Jeongup, Republic of Korea and* [2]*Battelle Korea, Seoul, Republic of Korea.*

Cell viability test is most commonly used and simple method for toxicity evaluation of materials like chemicals. In the study of nano-toxicity, its dispersibility has a big effect on the results, but still a lot of unknown facts remain behind veil. Two-crystal phases of titanium dioxide (TiO2, anatase and rutile) were used in this study in order to compare dispersibility and cytotoxicity with respect to crystal phase. FBS were used to control and vary the dispersibility of nanomaterial in cell culture media (RPMI 1640). Optical multiple scattering method was used to evaluate the homogeneity and stability. Firstly, each TiO2 was suspended in cell culture medium without or with FBS, for estimation of dispersion stability according to the medium condition and crystal phase. Both types of TiO2 show better stable dispersion with FBS than without FBS, and anatase is better dispersion than rutile in both medium conditions. Next, for evaluation of cytotoxicity with difference of dispersion stability, we measured cell viability after treatment with dispersed two-types TiO2 in each medium condition into A549 cells, human type II lung epithelial cell. The results show anatase < rutile in medium condition without FBS, but rutile > anatase after improvement of dispersion stability by FBS addition.
Taken together, our results indicate that dispersibility is different with their crystal phase and dispersion conditions. And cytotoxicity results is presented differently by their dispersibility. That is, dispersibility must be considered seriously for the cytotoxicity evaluation of nanomaterial.

PS 1481 SILICA NANOPARTICLES CAUSE GENOTOXIC ACTIVITY IN THE *IN VIVO* COMET AND MICRONUCLEUS ASSAYS WHILE GOLD OR POLYSTYRENE NANOMATERIALS DO NOT.

S. Pfuhler, T. Downs, M. Crosby, J. Jester, A. Allemang and K. Sarlo. *Central Product Safety, Procter & Gamble, Cincinnati, OH.*

Nanomaterials have unique properties and their genotoxic potentials are not fully understood; this causes concern regarding the potential impact on human health. It has been speculated that nanomolecular size, itself, leads to cellular alterations that can induce DNA damage. In an effort to understand potential modes of action (MoA) and bridge the work with in vitro assays, we tested nano/microsize gold particles (2, 20 and 200nm), latex nano/microspheres (20 and 1000nm) and nanosize, amorphous silica (15nm; Levasil® 200) in a comet assay/micronucleus combination study. We measured DNA damage in liver, lung and blood lymphocytes and micronuclei in circulating reticulocytes after 3 consecutive iv injections to male Wistar rats (n=4-8) at 48, 24 and 4h before sacrifice. The vehicle control animals were injected with PBS or FBS while the positive controls received 25mg/kg bw cyclophosphamide at 48 and 24h, as well as 300mg/kg ethyl methanesulfonate at 4h before sacrifice. Isolated cells were investigated for DNA damage after lysis and electrophoresis under alkaline conditions and blood samples were also scored for micronuclei using flow cytometry. The results demonstrated a lack of genotoxic potential for all tested sizes of gold and latex particles in all parameters/tissues investigated. The 15nm size silica particles, however, did increase the number of micronucleated reticulocytes from 0.12±0.03% in the vehicle group to 0.22±0.08% at the highest dose tested (50mg/kg), while 25mg/kg of the 15nm silica particles did not cause an increase (0.10±0.02%). These results were corroborated by findings in the comet assay, as a statistically significant increase in DNA damage was observed in cells from the liver at the high dose but not the low dose of Levasil® 200.
The results obtained in this study support the conclusion that the nanosize of the particles, alone, does not trigger genotoxic activity. Evaluation of in vitro testing, as well as MoA studies related to the effect observed with amorphous silica, are ongoing.

PS 1482 USE OF MICROCOSMS TO EVALUATE FATE AND EFFECTS OF NANOMATERIALS IN AQUATIC SYSTEMS.

D. S. Barber, J. Gao, L. C. Smith and K. W. Powers. *University of Florida, Gainesville, FL.*

The database on potential effects of nanomaterials in aquatic organisms is growing, but most data is for single species toxicity tests and little information is available on the fate and effects of nanomaterials in more complex models of the aquatic environment. To address this, the distribution and effects of 12nm colloidal nanosilver, 25nm powdered nanosilver and 50nm nanoaluminum were examined. Microcosms contained artificial sediment as well as algae (P. subcapitata), primary consumers (C. dubia), epibenthic organisms (H. azteca) and endobethic organisms (T. tubifex). Microcosms were treated by adding nanomaterials to overlying water. Algal cell number, organism number, and metal distribution were quantified. The form of nanosilver added affected distribution in the microcosm. 7 days after the second weekly addition of 2 ug/L of colloidal nanosilver, 1.3 ± 0.4 ug/L of total silver was present in the water column though essentially none of this present as free dissolved silver. 41% of added silver was present in the water column, 23% was loosely associated with sediment or pore water, 12% was tightly bound to sediment, and 2.5% was associated with the microcosm container. Addition of aqueous suspensions of powdered nanosilver resulted in 7%, 47%, and 30% of the dose present in the water column, loosely sediment associated and tightly sediment associated fractions, respectively. Concentrations of silver in H. azteca and C. dubia treated with colloidal silver were 20 and 3-fold greater than control, respectively, however concentrations of silver in T. tubifex were unaltered. This silver exposure did not significantly alter survival or reproduction in C. dubia, though in standard acute toxicity tests would have resulted in near 100% mortality. These studies demonstrate that the complexity present in microcosm experiments can substantially alter the toxicity and fate of nanomaterials in the aquatic toxicity experiments. This work was supported by grants from the US Air Force Research Laboratory and NSF (BES0834075).

PS 1483 IMPACT OF DISSOLVED ORGANIC MATTER ON THE TOXICITY OF TITANIUM DIOXIDE NANOPARTICLES IN ZEBRAFISH EMBRYOS.

S. Yang[1], O. Bar-Ilan[2], K. M. Louis[3], R. E. Peterson[2], W. Heideman[2], R. J. Hamers[3] and J. A. Pedersen[1]. [1]*Molecular and Environmental Toxicology, University of Wisconsin Madison, Madison, WI,* [2]*School of Pharmacy, University of Wisconsin Madison, Madison, WI and* [3]*Department of Chemistry, University of Wisconsin Madison, Madison, WI.*

Applications of titanium dioxide (TiO2) nanoparticles (NPs) now range from cosmetics to photovoltaics because TiO2 can generate reactive oxygen species (ROS) upon adsorption of ultraviolet light. With predicted environmental concentrations nearing predicted no effect concentrations, concerns about adverse environmental effects of TiO2 NPs are increasing. Dissolved organic matter (DOM), ubiquitous in aquatic environments, can alter NP suspension stability impacting their environmental fate and bioavailability. Several studies have shown that DOM is both a source and sink of ROS in aquatic environments. We tested the hypothesis that DOM increases TiO2 NP toxicity by increasing TiO2 NP body burden through enhanced suspension stability and by increasing ROS generation in the presence of simulated sunlight. We examined the effect of Suwannee River humic acid (SR-HA), fulvic acid (SR-FA), and unfractionated natural organic matter (SR-NOM) on bare TiO2 NP toxicity to zebrafish (Danio rerio) embryos in the dark and simulated sunlight. Embryos were exposed to graded concentrations of Degussa TiO2 NPs alone or with 30 mg L-1 DOM for 5 d. Toxicity was assessed in terms of lethality and incidence/severity of gross malformations. All DOM compositions decreased aggregate size in the dark over the 24 h exposure period. In simulated sunlight, photocatalytic degradation of DOM by TiO2 was observed after 2-4 h exposure resulting in increased aggregate size. In the dark, an increase in lethality of TiO2 NPs was observed at all concentrations in the presence of SR-HA and several concentrations with SR-FA and SR-NOM. In simulated sunlight, a similar trend was observed with TiO2 LC50s shifting from 310 mg L-1 for TiO2 alone to 193, 250, and 265 mg L-1 with SR-HA, SR-FA, and SR-NOM, respectively. The effect of DOM on NP body burden and ROS generation will also be discussed.

PS 1484 NANOTOXICOLOGY AND NANOHEALTH BIBLIOMETRY FROM 2000 TO 2009.

W. Waissmann[1], M. M. Alencar[2], M. Moura[1], A. B. Veggi[1], T. Pastorello[1] and R. B. Santana[1]. *[1]Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation, Rio de Janeiro, Rio de Janeiro, Brazil and [2]Institute of Scientific and Technological Communication and Information in Health, Oswaldo Cruz Foundation, Rio de Janeiro, Rio de Janeiro, Brazil.* Sponsor: E. Silbergeld.

a) Scope: To know the number of published articles in the field of nanoscience and nanotechnology (NN) and what proportion of them are devoted to health and toxicology. b) Research approach: Initially, we looked for the total number of articles published in the field of NN, from January 1, 2000 to December 31, 2009. To this, we used a technique based on a complex strategy of keywords combination which could identified almost 530,000 articles. After known the total, we focused on three scopes of interest to public health, related to the journal categories of Web of Science (WoS). This database classifies journals in 232 categories. For the subject Food/Nutrition, 8 categories were selected, for Health, 61 categories, and for Toxicology, 1 category. c) Results: We identified 2229 articles from 87 countries and 268 different periodicals, which correspond to the combined scopes NN + Food / Nutrition, and 33083 articles, from 116 countries, attending the combination of NN + Health. For toxicological purposes, 1240 articles were identified that matched the scopes of NN + Toxicology, 33 that matched scopes NN + Food/Nutrition + Toxicology, and 514 articles that matched scopes NN + Health + Toxicology. The analysis of the annual profiles for all scopes shows rising number of published articles, in particular since 2007. The big difference between the total number of articles published in the area devoted to NN + Toxicology has been changing slowly if compared to the total number of articles published. From 2000 to 2003, the NN + Toxicology scope accounted for 0.16% of the total and in 2009 to 0.41% (in general, 0.23% for the period). d) Conclusions: The challenges that nanotechnology and nanomaterials bring to toxicology and their potential environmental and social impacts cannot be adequately addressed with the maintenance of the differences between NN and NN + Toxicology.

PS 1485 DISRUPTION OF MUSCARINIC RECEPTOR MEDIATED SIGNAL TRANSDUCTION BY OXIDATIVE STRESS.

C. Wu, J. Erickson, H. Wang, Y. Huang and R. S. Aronstam. *Biological Sciences, Missouri University of Science and Technology, Rolla, MO.*

The ability of metal oxide nanoparticles (20-60 nm) to alter muscarinic receptor signaling was studied in CHO cells stably transfected with the gene for human M3 muscarinic acetylcholine receptor. Activation of M3 muscarinic receptors induced a biphasic increase in [Ca2+]i: an initial, IP3-mediated release of Ca2+ from endoplasmic reticulum (ER) stores followed by a sustained phase of Ca2+ entry (i.e., store operated calcium entry, SOCE). The cells were exposed to the nanoparticles (0 – 100 μg/ml) 2.5 or 24 hours. Little toxicity was evident after the 2.5 hour exposure. Following a 24 hour exposure, however, the following order of toxic potency was obtained: CuO > ZnO = CoO > Cr2O3>NiO >>SiO2, TiO, Fe2O3. The different particles had multiple effects on calcium metabolism and muscarinic signaling: 1) resting calcium concentration was increased; 2) thapsigargin-induced release of calcium from the ER was depressed; 3) IP3 receptor-mediated release of calcium from the ER was inhibited; and 4) SOCE entry was decreased. Only ZnO, CuO, CoO and NiO particles consistently increased resting ([Ca2+]i. Thapsigargin induced depletion of ER calcium was diminished by exposure to ZnO, CoO and Mn2O3 particles. The initial (IP3 receptor-mediated) increase in [Ca2+]i induced by muscarinic stimulation was depressed only by exposure to ZnO. However, SOCE was depressed by several metal oxide particles, with the following order of potency (2.5 hour exposure): ZnO > CoO > NiO > Fe2O3. Thus, ZnO, CuO, CoO nanoparticles possessed the most cytotoxicity, and this toxicity was most closely related to changes in [Ca2+]i. ZnO depressed most aspects of calcium signaling in response to muscarinic stimulation. While several metal oxide nanoparticles interfered with SOCE, this effect did not correlate highly with nanoparticle toxicity. In previous studies, we related ZnO cytotoxic and signaling effects to induction of oxidative stress. The present findings raise the possibility that the two sets of action have different origins.

PS 1486 SYSTEMATIC EVALUATION OF THE TOXICITY OF CARBON-COATED AND NON-COATED COPPER AND NICKEL NANOPARTICLES.

S. Minocha, A. Sarangi, A. Tropsha and R. J. Mumper. *UNC Eshelman School of Pharmacy, Chapel Hill, NC.* Sponsor: A. Ghio.

Manufactured Nanoparticles (MNPs) offer unique advantages of small size and high surface area and are consequently applicable in a variety of areas ranging from the medical, agricultural to the electronics industry. In light of the paucity of information on the toxicity of these MNPs and their immense applicability, there is a current need to systematically study the toxicity profiles of MNPs. In this direction, we have evaluated the effect of carbon coating on the toxicity profiles of matched pairs of carbon-coated (C-Cu and C-Ni) and non-coated copper and nickel (Cu and Ni) MNPs in cell-based assays using the A549 alveolar epithelial cell line. MNPs were characterized for their size, zeta potential, morphology, surface composition, cellular localization and quantitative cell uptake. Dose dependent toxicity of MNPs was evaluated using the mitochondrial function and membrane integrity assays at 24 hr. A kinetic analysis to further investigate the mechanism of cell death was also performed using Annexin V/ PI staining assay. Average particle size measured by dynamic light scattering showed that all MNP sizes were on an average 20-fold higher than provided by the manufacturer. The TEM results confirmed the presence of MNP agglomerates. XPS analysis revealed oxidation of bare metal non-carbon-coated MNPs. Protection against oxidation is conferred by the carbon coating in C-Cu and C-Ni MNPs. Ni MNPs appear to be benign under the tested conditions and C-Ni MNPs altered the mitochondrial function. Cu and C-Cu MNPs showed altered mitochondrial function and perturbed membrane integrity. Annexin V/PI staining assay for Cu and C-Cu MNPs suggest that cell death may occur through a necrotic pathway. In Conclusion the Cu and C-Cu MNPs were found to be more toxic than Ni and C-Ni MNPs. Currently we are investigating the physicochemical characteristics responsible for differential toxicity of Cu and C-Cu MNPs. This research is supported by a grant to RJM and AT from SRC/SEMATECH Engineering Research Center for Environmentally Benign Semiconductor Manufacturing.

PS 1487 THE OCCUPATIONAL HEALTH SURVEY ON WORKERS AND NANOPARTICLE NUMBERS IN THE FACTORY HANDLING NANO-TITANIUM DIOXIDE.

S. Ichihara[1], W. Li[2], T. Kobayashi[3], S. Omura[3], Y. Hiraku[5], X. Ding[2] and G. Ichihara[4]. *[1]Life Science Research Center, Mie University, Tsu, Japan, [2]Shanghai Institute of Planned Parenthood Research, Shanghai, China, [3]Tokyo Institute of Technology, Yokohama, Japan, [4]Nagoya University Graduate School of Medicine, Nagoya, Japan and [5]Mie University Graduate School of Medicine, Tsu, Japan.*

Background: Nano-titanium dioxide is widely used as pigment, sunscreen, and photocatalyst. Although several epidemiological studies were reported, there was no information on the particle size. Past epidemiological studies were on the workers producing titanium dioxide, but there are no studies on workers handling them. The present study investigated possible respiratory and cardiovascular effects of titanium dioxide on the workers in Shanghai and the number of particles using condensation particle counter (CPC) and optical particle counter (OPC). Methods and results: The diameter of the individual particles before and during working was measured by electron microscopy. Whereas the first quarter of the primary particle size was bigger than 100 nm before working, the value was less than 100 nm during working at the first and second days. Size dependent particle number concentration was measured by a combined CPC-OPC instrument. The number concentration of particles with a dimension more than 10 or 5 μm was correlated with the number concentration of particles with a dimension less than 100 nm, suggesting that the particle aggregation occurred in the workplace. The mobile ECG was attached to eight workers and heart rate was monitored during work. In some workers heart rate was correlated with the number concentration of particles with a dimension with a dimension less than 100nm. Conclusion: Given that the aggregation of nano- titanium dioxide plays an important role in the environmental effects of nanoparticles, it is essential to analyze the status of particles in workplace to consider the risk for the workers.

PS 1488 QUANTITATIVE PROTEOMICS ANALYSIS OF ADSORBED PLASMA PROTEINS CLASSIFIES NANOPARTICLES WITH DIFFERENT SURFACE PROPERTIES AND SIZE.

H. Zhang, K. E. Burnum, M. L. Luna, B. O. Petritis, J. Kim, W. Qian, R. J. Moore, B. Webb-Robertson, R. D. Smith, B. D. Thrall, J. G. Pounds and T. Liu. *Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA.*

The biological activity, biocompatibility, disposition and fate of engineered nanomaterials can be directly affected by layers adsorbed proteins or "protein corona". We on the interactions between human blood plasma proteins and nanoparticles with a controlled systematic variation of properties using stable isotope labeling and liquid chromatography-mass spectrometry (LC-MS) based quantitative proteomics. We developed a novel protocol to both simplify isolation of nanoparticle bound proteins and improve reproducibility. LC-MS analysis identified and quantified plasma proteins associated with polystyrene nanoparticles consisting of three different surface chemistries and two sizes, as well as, for four different exposure

times (for a total of 24 different samples). Quantitative comparison of relative protein abundances was achieved by spiking an 18O-labeled "universal reference" into each individually processed unlabeled sample as an internal standard, enabling simultaneous application of both label-free and isotopic label quantification across the sample set. Clustering analysis of 42 plasma proteins that presented significance quantitative proteomics difference dependent on surface modifications of nanoparticles resulted in distinctive patterns that classified the nanoparticles based on their surface properties and size. In addition, temporal data indicated that the formation of the stable protein "corona" that was isolated for the quantitative analysis was at equilibrium within 5 min. The comprehensive quantitative proteomics results obtained in this study have potential implications towards predicting nanoparticle biocompatibility. Portions of this research were supported by the Pacific Northwest National Laboratory Directed Research Development program, ES016212, and RR018522.

PS 1489 EFFECTS OF CERIUM OXIDE NANOPARTICLES ON *GLYCINE MAX* GROWN IN HYDROPONICS.

A. De La Rosa[1], J. R. Peralta[1], J. Gardea-Torresdey[1] and J. Terry[2]. [1]*Chemistry, University of Texas at El Paso, El Paso, TX and* [2]*Physics, Illinois Institute of Technology, Chicago, IL.*

Cerium oxide nanoparticles (NP) have a high level of UV absorption and antioxidant behavior, making them great candidates for sun screens and beauty products. Nanoceria have also been found useful in treating cancer and glaucoma attributed to their antioxidant properties. The increased use of these NP underscores the importance of understanding the environmental fate and toxicity. So far, information about nanoceria toxicity to plants is scarce. In this study, soybean plants were treated for 14 days in a modified Hoagland solution containing varying concentrations of CeO2 NP (0, 500, 1000, 2000, and 4000 mg/L). The objectives are to determine the effect of CeO2 NP on plant growth as well as monitor the uptake, deposition, and biotransformation of CeO2 NP. Leaves, stems, and roots of the treated soybean plants were analyzed separately using ICP-OES and X-ray absorption spectroscopy (XAS). The ICP-OES results showed cerium concentrations in roots varying from 37068- 74774 mg kg-1 for the treatments varying from 500- 4000 mg CeO2 L-1. In soybean stems, the highest accumulation occurred at 4000 mg L-1, with a cerium concentration of 3028 mg kg-1. While in soybean leaves, the highest accumulation occurred at 1000 mg L-1 with a cerium concentration of 1048 mg kg-1. In soybean stems and roots, the cerium concentration increased as the external concentration increased. The XAS results suggest that soybean absorb and store the nanoceria without any biotransformation. Future directions include the use of transmission electron microscopy to determine the location of the nanoparticles within the soybean tissues; therein determining the mechanism by which the uptake and deposition of nanoparticles occurs.

PS 1490 EFFECTS OF 12-WEEK-INHALATION EXPOSURE TO ETHYL TERT-BUTYL ETHER (ETBE) ON BEHAVIOR AND BRAIN IN C57BL/6J AND ALDH2(-/-) MICE.

R. Hojo, H. Kubota, M. Suda, Y. Yanagiba and R. Wang. *JNIOSH, Kawasaki, Kanagawa, Japan.* Sponsor: C. Tohyama.

We studied the possible effects of inhalation exposure to Ethyl tert-butyl ether (ETBE) on emotional and motor behavior, and brain histology in C57BL/6J (wild type, WT) and Aldh2(-/-) (knock out, KO) mice. Female and male C57BL/6J and Aldh2(-/-) mice in testing groups were exposed to either one of 0, 500, 1750 or 5000ppm of ETBE with inhalation for 12 weeks (6 hours/day, 5 days/week). Openfield test was conducted to examine emotion and activity of mice after ETBE exposure. Rota rod test was also induced for measuring motor function and motor coordination of mice. On the last day of these behavioral tests, animals were euthanized with overdose of anesthetic. Specimens of the brains were examined for histological modification with Immunohistochemistry staining. The c-fos positive cells in selected areas of the brain, forebrain, hippocampus, striatum, accumbens, and amygdale, of exposed-animals were counted and compared with animals from the control group. The results in behavioral tests indicated that ETBE inhalation affected on both tests, but only for male animals. In the openfield test, activity of exposed animals in both WT and KO showed tendency of decreased activity with a dose-dependent manner. In the rota rod test, motor function of exposed groups were significantly lower than that in control group, again only in male. In the brain histological examination, the numbers of c-fos positive cells in all brain areas did not show clear differences between exposed and control animals. The results of this study indicated that there were some toxic effect on the behavior but brain histology. ETBE inhalation has possible toxic effects to differently affect with sex. Male animals showed higher sensitivity to ETBE.

PS 1491 OXIDATIVE STRESS ASSOCIATED WITH ULTRAFINE MODEL PARTICLE SYSTEMS CONTAINING CHEMISORBED ENVIRONMENTALLY PERSISTENT FREE RADICALS.

M. A. Kelley[1], V. Y. Hebert[1], S. A. Cormier[2], B. Dellinger[3], S. Lomnicki[3] and T. R. Dugas[1]. [1]*Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, LA,* [2]*Pharmacology & Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA and* [3]*Chemistry, Louisiana State University, Baton Rouge, LA.*

Particulate matter (PM) is often emitted during thermal processes such as combustion of fuels and wastes. Exposure to PM has been associated with exacerbation of pulmonary and cardiovascular diseases. Oxidative stress may play a role in their mechanism of toxicity. Aromatic compounds often chemisorb to the surface of metal-oxide-containing PM following combustion, resulting in formation of surface-stabilized environmentally persistent free radicals (EPFR). Our objective was to investigate whether model EPFRs, synthesized as surrogates for PM formed during combustion, redox-cycle to generate reactive oxygen species (ROS). ROS production of model EPFRs was measured in silico by incubation with a fluorescent probe. ROS production was markedly increased for chemisorbed particle systems. Moreover, ROS levels were significantly elevated in Hep-2 cells exposed to EPFR. To validate these findings, hydroxyl radical generation was measured using an HPLC-based scavenging assay. Hydroxyl radical levels were elevated in EPFR incubated in silico and were further increased in the presence of thiols or ascorbate (AA). Further, hydroxyl radical production was also detected in EPFR incubated together with bronchoalveolar lavage fluid (BALF), verifying that EPFR-mediated ROS production is maintained in biological fluids. Finally, the effect of chronic exposure of neonatal rats to EPFR was examined. AA levels in plasma and BALF of exposed rats were measured to indirectly assess EPFR-induced oxidant production. Although plasma AA levels in treated rats were comparable to controls, levels of AA in BALF were elevated nearly 3.5-fold by particle exposure, suggesting a possible compensatory response to EPFR-mediated ROS production. P42ES013648.

PS 1492 IDENTIFICATION OF SYSTEMIC MARKERS FROM A PULMONARY CARBON NANOTUBE EXPOSURE.

T. Hulderman, A. L. Liston, R. Salmen-Muniz, S. H. Young, P. C. Zeidler-Erdely, V. Castranova, P. P. Simeonova and A. Erdely. *NIOSH, Morgantown, WV.*

Currently there is expanding interest, from the perspectives of occupational health surveillance and future epidemiological research, in early monitoring of worker exposure to engineered nanomaterials. Here, we highlight quantitative systemic markers in mice after a single exposure to carbon nanotubes (CNT) (multi-walled (MW) CNT or single-walled (SW) CNT). Mice were exposed by pharyngeal aspiration to 40μg and harvested at 24 hr, 7 and 28d post exposure. Mice were sacrificed and the following parameters were analyzed: whole blood gene expression, blood and bronchoalveolar lavage (BAL) differentials, serum protein profiling by immunoplex and proteomics, and gene expression analysis in the lung and extrapulmonary tissues including aorta, heart and liver. Early effects of pulmonary CNT exposure include a rapid but transient increase in inflammatory blood gene expression (IL-1β, CXCL2) and serum cytokines (IL-6, IL-5, CCL11). This was followed by an acute phase response including CRP, SAA-1, SAP and haptoglobin as shown by elevated serum protein levels and liver gene expression. Beyond 24hr there was a consistent increase in both blood and BAL eosinophils. These data correspond well with early increased serum IL-5 and CCL11 after MWCNT exposure. At 28d, serum acute phase proteins with immune function including complement C3, apolipoprotein A-I and A-II, α2-marcoglobulin, serotransferrin and liver carboxylesterase N were increased. The systemic markers correlated well with existing literature showing end-point measurements of pulmonary CNT exposure and adverse cardiovascular effects and enhanced allergic response.

PS 1493 *IN VITRO* VASCULAR TOXICITY OF METAL OXIDE NANOPARTICLES.

M. Odegaard and K. Dreher. *Environmental Public Health Division, U.S. EPA, Research Triangle Park, NC.*

Engineered nanoparticles (NPs) are designed to possess unique physicochemical properties, but may also produce atypical and unforeseen exposure scenarios with adverse health effects. The ability of NPs to translocate into systemic circulation following either inhalation or ingestion makes the vascular system a prime target for potential adverse health effects. The purpose of the current studies was to screen a variety of commercially-available TiO2 (6 types) and CeO2 (2 types) NPs varying in size and composition for vascular endothelial cell (EC) toxicity. A moderate-

throughput in vitro approach was used to determine effects on EC viability, cytotoxicity, and oxidative stress. Because ECs derived from different anatomical locations display substantial heterogeneity, three different types of primary human ECs were used for these studies: coronary artery (HCAEC), aortic (HAEC), and lung microvascular cells (HMVEC-L). NP dispersion conditions were tested and optimized, and ECs were exposed overnight to a range of doses (6.25-200 ug/ml). NP exposure resulted in significant dose-dependent decreases in cell viability and increases in cytotoxicity (LDH release). Effects were independent of particle size, while HCAEC and HAEC cells showed greater sensitivity than HMVEC-L cells. All three cell types showed similar effects of NP exposure on oxidative stress, with slightly greater sensitivity in the HAECs. Importantly, oxidative stress was most strongly increased after exposure to TiO2 but not CeO2 NPs, and was not directly correlated with changes in viability or cytotoxicity. In summary, these data reveal that exposure of human endothelial cells to metal oxide NPs results in gross detrimental health effects. Overall, the observed effects were particle, cell-type and endpoint-specific. Our approach of directly comparing different commercially-available NPs in vitro provides a useful screening system for assessing health effects and prioritizing future in vivo toxicity testing, as well as identifying alternative test methods to predict NP vascular toxicity. This abstract does not reflect EPA policy.

PS 1494 ACUTE PHASE PROTEINS AS BIOMARKERS FOR PREDICTING THE EXPOSURE AND SAFETY OF NANOMATERIALS.

Y. Yoshioka[1, 2, 3], K. Higashisaka[1, 3], K. Yamashita[1, 3], M. Fujimura[1, 3], Y. Morishita[1, 3], P. Huiyan[1, 3], T. Ogura[1, 3], H. Nabeshi[1, 3], K. Nagano[3], Y. Abe[3], H. Kamada[2, 3], S. Tsunoda[2, 3, 4], N. Itoh[1, 3], T. Yoshikawa[1, 3] and Y. Tsutsumi[1, 2, 3]. *[1]Department of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan, [2]The Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka, Japan, [3]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, Osaka, Japan and [4]Department of Biomedical Innovation, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan.*

Recently, nanomaterials have become an integral part of our daily lives. However, current knowledge of the potential risk of nanomaterials is considered insufficient. Here, we attempted to identify biomarkers for predicting the exposure and safety of nanomaterials by using a proteomics based approach. We evaluated the changes of protein expression in plasma after intravenous injection of nanosilica particles. Our analyses identified haptoglobin, one of the acute phase proteins, as a candidate biomarker. The results of ELISA showed that the level of haptoglobin was significantly elevated in plasma of mice exposed to nanosilica particles with a diameter of 70 nm (nSP70) compared to normal mice and those exposed to silica particles with a diameter of 1000 nm. Furthermore, the other acute phase proteins, C-reactive protein (CRP) and serum amyloid A (SAA) were also elevated in plasma of nSP70 treated mice. In addition, the level of these acute phase proteins was elevated in the plasma of mice after intranasal treatment with nSP30. Our results suggest that haptoglobin, CRP and SAA are highly sensitive biomarkers for assessing the exposure to nanosilica particles. We believe this study will contribute to the development of global risk assessment techniques for nanomaterials.

PS 1495 TOLL-LIKE RECEPTOR 4 IS INVOLVED IN THE CEREBROVASCULAR TOXICITY OF PCB153 BOUND TO NANOPARTICLES.

B. Zhang[1, 2], L. Chen[2], B. Hennig[1, 3] and M. Toborek[1, 2]. *[1]Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY, [2]Department of Neurosurgery, University of Kentucky, Lexington, KY and [3]College of Agriculture, University of Kentucky, Lexington, KY.*

Polychlorinated biphenyls (PCBs) are organochlorinated chemicals that persistent in the environment due to their structural stability and high lipophilicity. PCBs have adverse effects on human health, including vascular disorders and stroke. PCBs released from environmental sources are capable of binding onto nanoparticles (NPs) present in the environment and being taken up by humans. However, the toxicity of PCBs assembled onto nanoparticles is unknown. In the current study, we coated silica nanoparticles (20 nm diameter size) with PCB153. Mice (C57BL/6, males, 12-week-old) were injected with PCB153 bound to nanoparticles (PCB153-NP), PCB153, nanoparticles (NP) alone, or vehicle (PBS). PCB153 was administered in the amount of 5 ng/g body weight. Exposure to PCB153-NP but not to other treatment factors resulted in the loss of the integrity of the blood-brain barrier (BBB) as determined by increased flux of sodium fluorescence from brain microvessels into the brain tissue. To investigate the mechanisms of the cerebrovascular toxicity of PCB153-NP, toll-like receptor 4-deficient (C3H/HeJ) and control (C3H/HeOuJ) mice (males, 10-12 weeks old) were exposed to PCB153-NP and control treatments. Expression of tight junction protein ZO-1 was significantly decreased in animals with normal TLR4 expression exposed to PCB153-NP. However, this effect was not detected in TLR4-deficient mice. In addition, treatment with PCB153-NP induced overexpression of proinflammatory cytokines in control but not in TLR4-deficient mice. Our data indicate that exposure to PCB153-NP results in strong proinflammatory responses in the central nervous system and can disrupt the integrity of the BBB via alterations of tight junction protein expression. More importantly, deficiency of TLR4 may attenuate the toxicity of PCB153-NP in cerebral vasculature. Supported by P42 ES 07380.

PS 1496 ASSESSMENT OF PULMONARY TOXICITY IN MICE AFTER INHALATION EXPOSURE TO ZINC OXIDE.

A. Adamcakova-Dodd[1], J. Kim[2], S. U. Vorrink[2], L. V. Stebounova[3], P. T. O'Shaughnessy[1], V. H. Grassian[3, 2] and P. S. Thorne[1, 2]. *[1]Occupational and Environmental Health, University of Iowa, Iowa City, IA, [2]Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA and [3]Chemistry, University of Iowa, Iowa City, IA.*

Zinc oxide (ZnO) nanoparticles (NPs) are used in many industrial and consumer products (sunscreen, paints, coatings) thus pulmonary exposure to these materials can occur when NPs are aerosolized. Our aim was to assess pulmonary toxicity of ZnO after sub-acute inhalation exposure using mouse model.
Commercially available ZnO NPs (Meliorum) with primary particle size 15 nm were used. Male mice (C57Bl6/J) were exposed to ZnO aerosol for 4 hrs/day, 5 days/wk for 2 or 4 weeks in a dynamic whole-body exposure chamber. Control (sham) mice were exposed to filtered laboratory air. Zinc oxide aerosol was generated into the exposure chamber using a Collison nebulizer followed by a heating tube and charge neutralizer. Average concentration of ZnO aerosol in the chamber was 3.6 mg/m3 and the median particle size diameter inside of the chamber was 63 nm indicative of some aggregation. One group of mice was necropsied immediately after last exposure (0 wk), another group 3 weeks (3 wks) after the last exposure. Bronchoalveolar lavage fluid (BALF) was analyzed for differential and total cells, total protein, activity of lactate dehydrogenase (LDH) and cytokines. Total and differential white blood cells in the blood were counted. Lungs were processed for histopathology and pulmonary mechanics measurements were taken (flexiVent). Zn content in the lungs, heart, liver, spleen, kidney and brain was determined. We performed dissolution studies in biological fluids.
Total number of cells in BALF was significantly increased in animals exposed to ZnO and necropsied at 0 wk post exposure in comparison with sham-exposed mice. Total cell counts returned to baseline 3 wks post exposure. There was very low neutrophilic response (1.7%) observed in ZnO-exposed mice at 0 wk. Overall Meliorum (ZnO NPs) did not show significant pulmonary toxicity.

PS 1497 DEVELOPMENT OF A MOUSE MODEL FOR PREDICTING DRUG HYPERSENSITIVITY REACTIONS.

A. Hudson, S. H. Cole, T. T. Kawabata and J. Whritenour. *Immunotoxicology, Pfizer Global R&D, Groton, CT.*

Animal models to determine the potential of drug development candidates to produce hypersensitivity reactions have not been adequately optimized, validated and characterized. The mouse lymph node proliferation assay (LNPA), developed as an alternative to the mouse popliteal lymph node assay, measures cell proliferation in the draining lymph nodes following subcutaneous drug exposure. Initial validation data from an inter-laboratory study of the LNPA looked promising (Weaver et al. J. Immunotoxicol. 2:11-20, 2005). The objective of this project was to further optimize and validate the mouse draining lymph node model utilizing a non-radioactive endpoint. Compounds that have been associated with hypersensitivity reactions in the literature (positive controls) were tested in the model in addition to compounds with few or no reports (negative controls) of hypersensitivity. Doses were selected based on clinical exposure data or on the highest dose achievable based on solubility and clinical side effects, including irritation. Mice received three subcutaneous injections of drug or vehicle into the scruff of the neck once daily for a period of three days. Following a three day rest period, draining lymph nodes were harvested and single cell suspensions prepared. Total and large lymphocyte cell numbers were determined by flow cytometry for each animal. The stimulation index was calculated by dividing the mean number of large lymphocytes for the treated group by that of the vehicle treated animals. Animals with a total large lymphocyte number >5X the mean of the vehicle group were classified as a "responder"

and the percentage of responders per group was determined. The percentage of responders ranged from 80-100 and 0-20 for the positive (ofloxacin, amoxicillin, sulfamethoxazole) and negative (metformin, acetaminophen, phenobarbital) control drugs, respectively, indicating that the model is able to differentiate between positive and negative controls. These data suggest that this model may be a useful tool for identifying compounds with the potential to produce hypersensitivity responses.

PS 1498 HLA-SPECIFIC T-CELL ACTIVATION AS AN UNDERLYING CAUSE OF DRUG-INDUCED HYPERSENSITIVITY.

J. M. Goodwin[1], T. Kawabata[2] and M. T. Pletcher[1]. [1]*Compound Safety Prediction, Pfizer, Groton, CT and* [2]*Immunotoxicology CoE, Pfizer, Groton, CT.*

Drug hypersensitivity is a major safety issue in drug discovery. Though it occurs in only a small fraction of the patient population, the results are often severe. Genome-wide association studies have been employed to better understand the underlying mechanisms and have shown an association between alleles of the major histocompatibility complex (MHC) region and an individual's hypersensitivity to certain drugs. One hypothesis that has been put forth to explain the genetic relationship between adverse events and the MHC is the Pharmacological Interactions (PI) concept. It states that certain drugs can bind to T-cell receptors, mimicking a ligand, resulting in T-cell activation in an MHC-dependent fashion. We sought to further investigate the validity of this hypothesis by determining a transcriptional response indicating T-cell activation in PBMCs with hypersensitive haplotypes. PBMCs with known reactive and non-reactive MHC haplotypes were dosed with their corresponding reactive drugs (HLA-B*5701 with Abacavir and HLA-B*1502 with Carbamazepine) and RNA was profiled on microarrays covering 172 genes associated with T and B cell activation. We identified 27 candidate genes, including IL-12beta, CD80, IL-7, IL-13, CXCR3, and CSF2, whose expression was altered only in the hypersensitive haplotype treated with reactive drug. We then examined the expression changes of these selected genes on a larger panel of PBMCs from patients with various non-reactive and reactive haplotypes to confirm the transcriptional fingerprint. In conclusion, we have identified a set of genes that are regulated in an MHC-dependent fashion in PBMCs treated with compounds known to induce hypersensitivity reactions in patients carrying these specific alleles. This identification of the genetic and transcriptional factors involved in the development of immune-mediated idiosyncratic tissue injury provides the insight necessary to develop diagnostic tools to identify patient populations at increased risk and new treatment strategies.

PS 1499 EARLY CHANGES IN AROMATIC AMINE-INDUCED IDIOSYNCRATIC DRUG REACTIONS.

W. Ng and J. Uetrecht. *Pharmacy, University of Toronto, Toronto, ON, Canada.*

Virtually all drugs that contain a primary aromatic amine group are associated with a high incidence of idiosyncratic drug reactions (IDRs) and this is considered a structural alert for drug development. This suggests that this functional group has biological effects that might be used as a biomarker to predict IDR risk as well as provide clues to the mechanism of IDRs. A previous microarray study to determine the early changes (12 h) in hepatic gene expression in Brown Norway rats treated with the aromatic amine drugs sulfamethoxazole (SMX, 150 mg/kg), dapsone (DDS, 20 mg/kg), and aminoglutethimide (AMG, 80 mg/kg) provided the basis for our present studies. The pattern of mRNA changes was significantly different for the 3 drugs. Three distinct changes from the microarray data were pursued in terms of their downstream effects: early insulin-induced gene (Eiih), chemokine (C-X-C) 1 (Cxcl1), and genes involved with the Nrf2-ARE pathway. PCR analysis of Eiih confirmed that it was up-regulated at 12 h in the liver with all drugs tested, although the change was greater for AMG and DDS than SMX. The function of Eiih is presently unknown but these data suggest that it could be a potential biomarker of aromatic amine-induced IDRs. Despite moderate changes in hepatic Cxcl1 and an up-regulation of Cxcl1 protein levels in the blood of AMG-treated rats this was not the case for SMX- or DDS-treated animals. This increase in Cxcl1 coincided with a transient increase in peripheral blood neutrophils 24 h after AMG treatment. This finding may be related to the agranulocytosis that sometimes occurs in AMG-treated patients. Up-regulation of the Nrf2-ARE pathway was also observed: increased glutathione-S-transferase and thioredoxin reductase in the liver 48 h after AMG treatment. This suggests that these aromatic amines induce oxidative stress. These results demonstrate the diverse effects of aromatic amine drugs and illustrate the difficulties in finding a common biomarker that predicts IDR risk. This research was funded by grants from the Canadian Institutes for Health Research.

PS 1500 THE USE OF MAST CELLS DERIVED FROM HUMAN CD34+ HEMATOPOIETIC STEM CELLS AS A TOOL FOR PREDICTING PSEUDOALLERGIC DRUG REACTIONS.

J. Whritenour[1], C. Homiski[1], K. Haskell[2], M. Thiede[2] and T. T. Kawabata[1]. [1]*Immunotoxicology CoE, Drug Safety Research and Development, Pfizer, Inc., Groton, CT and* [2]*Genetically Modified Mice CoE, Pfizer, Inc., Groton, CT.*

Pseudoallergic (anaphylactoid) reactions are the result of direct mast cell activation and degranulation via an IgE-independent mechanism, resulting in the release of specific mediators such as histamine, tryptase, and cytokines. Assays to predict the potential of drugs to produce pseudoallergic reactions in humans are currently unavailable but would be a useful tool during drug development. In this study, we explored the utility of CD34+ hematopoietic stem cell (HSC)-derived mast cells for predicting the pseudoallergic potential of various drugs using histamine release as an indicator of mast cell degranulation. CD34+ HSC from human bone marrow donors were cultured over a period of ~68 days with various cytokines and differentiated into cells expressing a mast cell phenotype (FcεR1+, CD45+, CD117+). Mast cells were plated ($5x10^4$ cells/well) and incubated for 30 min, 60 min, or 24 hr with either vehicle (PBS), or compounds known (tubocurarine, morphine) or not (metformin, acetaminophen) to produce pseudoallergic reactions in humans. Following incubation, contents from each well were centrifuged to remove the cells and the supernatants were collected and analyzed for histamine content using a commercially available ELISA kit. While the overall magnitude of the histamine response appears to be donor-dependent, CD34+ HSC-derived mast cells consistently responded to the known pseudoallergens. Histamine levels were similar between cells treated with the negative controls and the vehicle. No significant differences were measured in the quantity of histamine released when mast cells were incubated with compounds for 30 min, 60 min, or 24 hr. These data indicate that human CD34+ HSC-derived mast cells may be used to predict for pseudoallergic potential of drug development candidates.

PS 1501 EFFECT OF RETINOIC ACID ON NEVIRAPINE-INDUCED SKIN RASH AND THE INVOLVEMENT OF TH17 CELLS.

X. Chen and J. P. Uetrecht. *Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.*

Introduction: Nevirapine (NVP) is used to treat AIDS but is associated with a high incidence of skin rash and liver toxicity. We found that it also causes a similar skin rash in the Brown Norway (BN) rats. The NVP-induced skin rash in BN rats is clearly immune-mediated and we think the mechanism is similar to that in humans. For example, a low $CD4^+$ T cell count decreases the risk of rash in both rats and humans. Furthermore, $CD4^+$ T cells can transfer the sensitivity from sensitized animals to naïve animals. We found that IL-17 was produced by lymphocytes from NVP-sensitized animals suggesting that IL-17 and/or Th17 cells might be involved in the pathogenesis. Retinoic acid (RA), a vitamin A metabolite, is reported to inhibit Th17 cells. In this preliminary study we examined the effect of RA on NVP-induced skin rash and the involvement of Th17 cells. **Methods and Results**: Female BN rats co-treated with NVP and RA for 21 days had no symptoms of NVP-induced idiosyncratic reactions, i.e. red ears and/or skin rash. However, slightly lower plasma levels of NVP and/or its metabolites were observed. In the peripheral blood, NVP treatment significantly decreased the percentage of cells that express $CD4^+/CD161^{low}$, which in humans is the precursor of Th17 cells. We also observed a big spike of $CD4^-CD161^{low}$ cells on day 4 following NVP treatment but they deceased to baseline levels in 1 week. These might be $CD8^+$ or NK cells and they need to be further characterized. **Conclusions**: The protective role of RA on NVP-induced skin rash might be a result of decreased drug plasma levels. However, this needs to be confirmed by using a higher dose of NVP or a P450 inhibitor with co-administration of RA. The involvement of Th17 cells ($CD4^+IL17^+$) will also be investigated using a more direct method, i.e. intracellular staining. Completion of these studies will provide a better understanding of the mechanism of NVP-induced skin rash and, in turn, this may provide possible ways to prevent and/or treat idiosyncratic drug reactions. This work was supported by grants from CIHR.

PS 1502 IMMUNOLOGICAL ASPECTS OF AN ANIMAL MODEL OF AMODIAQUINE-INDUCED LIVER TOXICITY.

J. Li[1], H. C. Workman[2], P. Cai[3], G. Kourteva[4], H. Hilton[4], H. Haggerty[1], D. Moore[2] and J. Uetrecht[3, 5]. [1]*Immunotoxicology, Bristol-Myers Squibb Company, New Brunswick, NJ,* [2]*Nonclinical Safety, F. Hoffmann-La Roche Inc., Nutley, NJ,* [3]*Pharmacy, University of Toronto, Toronto, ON, Canada,* [4]*TRS-RNA Biomarker Technology, F. Hoffmann-La Roche Inc., Nutley, NJ and* [5]*Medicine, University of Toronto, Toronto, ON, Canada.*

Rationale: As in humans, amodiaquine (AQ) causes a delayed onset liver injury in rats, but the ALT returns to normal despite continued treatment. This is consistent with immune tolerance and we tried to overcome this tolerance with IL-7 co-treatment. Experimental Procedures: Male Brown Norway rats were treated with AQ at 62.5 mg/kg/day p.o. for six weeks with IL-7 co-treatment at 100 ug/kg/day i.p. for 7 days during either the 2nd or 3rd week. Serum ALT and cytokines were measured weekly. Liver histopathology, splenocyte phenotype, hepatic mRNA expression, serum prolactin and serum microRNAs were determined at the end. Results: Liver findings include individually scattered activated Kupffer cells (KC) (vacuolated and clustered) and KC microgranulomas as well as hepatocyte single cell necrosis. This was slightly enhanced by both IL-7 co-treatment schedules. Macrophage and B cell activation was observed in the spleen. IL-7 given during the 3rd week appeared to increase the elevation of ALT while IL-7 during the 2nd week delayed it. There was a good correlation between serum levels of ALT and MCP-1. Hepatic mRNAs involved in apoptosis (i.e. caspase 1, Fas and Faslg) and immunological functions (i.e. CD3, IL-4, IL-18, CCL5, TLR 2, 4, 7) were up-regulated in all AQ groups, with prolactin receptor being most up-regulated (40-60 fold, $p < 0.0001$). IL-7 co-treatment caused more gene expression changes (i.e. TNF-α, IL-1β). Significantly elevated serum levels of prolactin and microRNA-122 were also detected in AQ-treated rats. Conclusions: Amodiaquine treatment for 6 weeks resulted in KC hyperplasia and single cell necrosis in the liver. The delayed onset and up-regulation of immune-related genes suggests that this injury is immune-mediated. This work was supported by CIHR grants and the Roche postdoctoral fellowship program.

PS 1503 CHANGES IN HEPATIC GENE EXPRESSION INDUCED BY AMODIAQUINE IN RATS.

J. Uetrecht[3, 5], J. Li[1], G. Kourteva[2], H. Hilton[2], P. Cai[3], H. C. Workman[4], H. Haggerty[1] and D. Moore[4]. [1]*Immunotoxicology, Bristol-Myers Squibb Company, New Brunswick, NJ,* [2]*TRS-RNA Biomarker Technology, F. Hoffmann-La Roche Inc., Nutley, NJ,* [3]*Pharmacy, University of Toronto, Toronto, ON, Canada,* [4]*Nonclinical Safety, F. Hoffmann-La Roche Inc., Nutley, NJ and* [5]*Medicine, University of Toronto, Toronto, ON, Canada.*

Rationale: Previous studies indicated that treatment of Brown Norway (BN) rats with amodiaquine (AQ) leads to activation of the immune system, especially in the liver. This study profiled changes in hepatic mRNA expression induced by AQ at early time points during the initiation of hepatotoxicity. Methods: (1) Male BN rats (n=4) were treated with AQ at 62.5 mg/kg/day p.o. for 7 days and hepatic mRNA expression related to apoptosis and immune activation was determined by qPCR array. (2) Male BN rats (n=4) were given a single dose of AQ at 200 mg/kg p.o. and global gene expression profile in the liver at 6 h was determined by an Affymetrix chip (rat genome 230 2.0). mRNA expression data were further analyzed by either on-line software for qPCR array or Partek for Affymetrix chip. Serum HMGB1 was also measured by ELISA. Results: AQ treatment for 7 days resulted in the up-regulation of several genes crucial for apoptosis such as Faslg, Bax, and Caspase 1 etc., and of immune-related genes such as CD2, CD3, IL-1b, IL-4, IL-18, CCL5, IFN-g, and TLR 2 etc. Although not significant between groups, prolactin receptor (prlr) was dramatically up-regulated in two AQ-treated rats. Global gene expression screening at 6 h identified only a few but interesting changes: Prlr (2.6-fold, $p<0.05$), Gadd45b (2.1-fold, $p<0.05$), and Hamp (-13.4-fold, $p<0.0001$). Also, serum HMGB1 was significantly elevated in AQ-treated rats. Conclusions: Apoptosis related changes in mRNA levels and HMGB1 blood concentrations at 6 h and day 7 when ALT is still in the normal range implied that AQ-induced apoptosis is an early event in the initiation of liver injury that occurs at 4 weeks and may be immune-mediated. This work is supported by CIHR grants and the Roche postdoctoral fellowship program.

PS 1504 COVALENT BINDING OF NEVIRAPINE *IN VITRO* AND *IN VIVO*.

A. Sharma and J. Uetrecht. *Pharmaceutical Sciences, University of Toronto, Toronto, ON, Canada.*

Rationale: Nevirapine (NVP) treatment is associated with significant idiosyncratic and immune-mediated skin rash and hepatotoxicity. The central hypothesis is that development of skin rash and liver toxicity are mediated by covalent binding of a NVP metabolite to protein. This study was designed to examine covalent binding of NVP and metabolites to tissues in various species. Methods: Anti-NVP antibody was produced in New Zealand White Rabbits and used in immunoblotting studies to detect covalent binding of NVP to liver, skin, and ear proteins. Samples were obtained from Brown Norway (BN) rats treated with NVP (150 mg/day) or various analogs in vivo, and from C57 or Cbl-b (-/-) mice treated with NVP in vivo (550 or 950 mg/day). In vitro incubations using hepatic microsomes or homogenized rat skin were also performed. Results: Hepatic covalent binding was significantly lower for 12-OH-NVP than NVP as expected. Covalent binding was observed in the liver and skin of NVP treated BN rats. Major modified bands in the skin appeared at 60K and 76K. Homogenized rat skin incubated with 12-NVP-sulfate produced a strong band at 60K, and two minor bands at 50K and 71K. Significant alanine transaminase (ALT) increase was observed in male and female Cbl-b (-/-) mice treated with NVP at the highest dose weeks 2 to 3; ALT levels resolved by week 5. Cbl-b (-/-) mice with high ALT showed the strongest hepatic covalent binding and marked hepatic pathological changes. These included multifocal necroinflammation and parenchymal collapse with inflammatory infiltrate. Conclusions: The reactive metabolite that led to hepatic covalent binding is likely a quinone methide formed by oxidation of the methyl group; however, it does not appear that this occurs in skin. It is more likely that covalent binding in the skin involves 12-NVP-sulfate. Cbl-b (-/-) mice may serve as a possible animal model for NVP-induced hepatotoxicity, but the only way to determine if covalent binding is involved is to overcome immune tolerance. This research was funded by grants from the Canadian Institutes of Health Research.

PS 1505 DANGER SIGNALS IN NEVIRAPINE-INDUCED SKIN RASH.

X. Zhang and J. Uetrecht. *Pharmaceutical Science, University of Toronto, Toronto, ON, Canada.*

Background: The HIV drug, nevirapine (NVP) is associated with a high incidence of skin rash and liver toxicity in patients. We developed an animal model of the skin rash in which female Brown Norway (BN) rats treated with NVP develop an immune-mediated skin rash similar to the rash in humans. The goal of this study was to determine if the early events of skin rash involve the production of danger signals in the skin. There is a high degree of covalent binding of NVP in the liver and therefore a danger signal may be produced there even though the animals do not develop overt liver toxicity. The effects of 12-OH-NVP, a metabolite of NVP which induced a skin rash at a lower dose than NVP, and DNVP, an analogue of NVP in which the methyl hydrogens are replaced by deuterium (this inhibits 12-OH-NVP formation and decreases the incidence of rash) were compared to those of NVP. Methods and Results: 6 and 12 hrs after treatment of female BN rats with NVP or one of its analogs, changes in gene expression in the ear and the liver were determined with Affymetrix gene chips. Some genes, e.g., Nr4a3, were highly up-regulated by NVP and 12-OH-NVP but not by DNVP treatment in the ear. In the liver, more genes were significantly changed at 6 hrs than at 12 hrs. ER-stress and immune-response related genes were significantly up-regulated by NVP and DNVP, while 12-OH-NVP induced other genes, including CD36, a class B scavenger receptor on macrophages. Conclusions: These results are consistent with the danger hypothesis. Future experiments will involve modification of these genes to determine their role in the induction of skin rash by NVP. This research was supported by grants from the Canadian Institutes of Health Research.

PS 1506 INVOLVEMENT OF T HELPER 17 (TH17) PATHWAY IN D-PENICILLAMINE-INDUCED AUTOIMMUNITY.

X. Zhu[1], J. Li[3] and J. P. Uetrecht[1, 2]. [1]*Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada,* [2]*Faculty of Medicine, University of Toronto, Toronto, ON, Canada and* [3]*Department of Immunotoxicology, R&D, Drug Safety Evaluation, Bristol-Myers Squibb Company, New Brunswick, NJ.*

Rationale: Clinical evidence suggests that most idiosyncratic drug reactions (IDRs) are immune-mediated. Penicillamine causes a variety of autoimmune syndromes in humans and treatment of BN rats with penicillamine also causes autoimmunity. This represents a good animal model for this one type of IDR. Previous experiments suggested that Th17 cells may be involved in penicillamine-induced autoimmunity, since its featured cytokines IL-17 and IL-22 were found to be elevated in animals that developed autoimmunity. The aim of this study was to further characterize the role of Th17 cells in this IDR model. Experimental Procedures: BN rats were given penicillamine (1.5 mg/ml in drinking water for 5 weeks). At the endpoint of the treatment, lymphocytes were isolated from the cervical lymph nodes of either control or penicillamine-treated animals. The presence of CD4+IL-17+ cells was evaluated by flow cytometry. After cell surface staining, intracellular staining was performed for the detection of IL-17. Retinoic acid (RA), which has been shown to inhibit Th17 cell development in vitro, was given with penicillamine to

examine its effects on the development of autoimmunity. Results: 4 out of 8 rats treated with penicillamine developed autoimmunity. The percentage of CD4+IL-17+ cell population was found significantly increased in the animals that developed autoimmunity (1.65±0.46%), compared with control and non-sick groups (0.27±0.08% and 0.29±0.09%, respectively). Surprisingly, all the animals that had been treated with RA and penicillamine developed autoimmunity and it was more severe. Conclusions: Our data provided additional evidence in support of Th17 cell involvement in penicillamine-induced autoimmunity. However, further studies should be carried out to reveal the effects of RA on the in vivo development of Th17 cells and penicillamine-induced autoimmunity. This work was supported by Canadian Institutes of Health Research.

PS 1507 INDOLES MITIGATE THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY ACTIVATION AHR AND ER, RECIPROCAL INDUCTION OF REGULATORY T CELLS AND TH17 CELLS, AND INDUCTION OF APOPTOSIS.

M. Rouse, M. Nagarkatti and P. Nagarkatti. *University of South Carolina, Columbia, SC.*

Indigo is one of the oldest dyes known to mankind and produced by fermentation of certain plants. More importantly, indigo has been used in traditional Chinese medicine. Diindolylmethane (DIM) and its precursor, Indole-3-carbinol (I3C), for example, are two cruciferous indoles that are used as dietary supplements. Both DIM and I3C have been shown to act as AhR ligands similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In the current study, we hypothesized that DIM and I3C may regulate the immune response through the activation of the AhR and ER, similar to another dietary supplement, resveratrol. We induced experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS) by injecting MOG peptide into C57BL/6 mice along with complete Freund's adjuvant and pertussis toxin. The results demonstrated that use of DIM and I3C in EAE mice was effective in not only delaying the clinical symptoms of paralysis, but also curtailed the overall severity. We observed that treatment with DIM and I3C caused reciprocal regulation of Tregs and Th17 cells. In addition, DIM and I3C treated EAE mice revealed a dramatic decrease in CD4+ T-cells in the brain, while preserving resident microglial cells. In a H3-incorporation assay, DIM and I3C demonstrated a pronounced ability to inhibit ConA activated T-cell proliferation. Cell cycle analysis further suggested that treatment of these indoles can lead to increased apoptosis and G0/G1 arrest. Together, these studies demonstrate that indoles act through multiple pathways to suppress EAE development.(Supported in part by NIH grants R01ES09098, P01AT003961, R01ES019313).

PS 1508 TNF RECEPTOR 2 (TNFR2)-DEFICIENT MYELIN OLIGODENDROCYTE GLYCOPROTEIN (MOG)-SPECIFIC T CELL RECEPTOR (TCR) TRANSGENIC MICE DEVELOP SPONTANEOUS AUTOIMMUNITY.

P. G. Miller[3], E. N. Sellers[3], R. S. Schondelmeyer[3], M. B. Bonn[4], J. K. Dey[4], J. A. Cascio[2], C. Franklin[2], H. Braley-Mullen[2], N. H. Ruddle[5] and S. C. McKarns[1,2]. [1]*Surgery, University of Missouri, Columbia, MO,* [2]*Molecular Microbiology & Immunology, University of Missouri, Columbia, MO,* [3]*Biochemistry, University of Missouri, Columbia, MO,* [4]*Biological Sciences, University of Missouri, Columbia, MO and* [5]*Biological & Biomedical Sciences, Yale University, New Haven, CT.*

Multiple sclerosis (MS) is an autoimmune disease characterized by inflammatory infiltration and demyelination in the central nervous system (CNS). There is no cure. Genetic and environmental factors have been implicated in a loss of self-tolerance in genetically susceptible individuals. Tumor necrosis factor-alpha (TNF) has dual pro-inflammatory and immunoregulatory properties with key physiological and pathological regulatory roles. TNF antagonists treat several autoimmune diseases including rheumatoid arthritis and Crohn's disease. Yet, despite clinical evidence implicating that TNF contributes to MS, anti-TNF therapies unexpectedly worsen MS. Given the "double-edge sword" potential of TNF to differentially regulate both neuro-protective and destructive responses, the inability of TNF antagonists to treat MS may represent variable TNF signaling. We have shown that TNFR2, but not TNFR1,-deficiency increases susceptibility to MOG-induced EAE. The objective of this research is to define the role of TNFR2 signaling on cytokine production and neuronal protection in CNS autoimmunity. Here, we report a high incidence of lethal spontaneous neurodegenerative autoimmunity in female C57BL/6 TNFR2-deficient, but not TNF-deficient, mice bred onto the MOG-specific TCR transgenic (2D2) background. We correlate disease incidence in TNFR2.KO mice with infiltration of MOG-specific CD4+ T effector cells into the CNS and decreased suppression of cytokines by FoxP3+ CD4+ T regulatory cells. Our data demonstrate that TNFR2 is essential for the function of T regulatory cells and that CNS autoimmune disease varies with TNF signaling. These results identify selective activation of TNFR2 as a potential effective drug target for MS.

PS 1509 ANTI-MESOTHELIAL CELL AUTOANTIBODIES ASSOCIATED WITH ASBESTOS EXPOSURE AND PLEURAL DISEASE.

L. S. Marchand[2], J. C. Pfau[1], S. Sophie[1] and E. A. Putnam[3]. [1]*Biological Sciences, Idaho State University, Pocatello, ID,* [2]*School of Medicine, University of Washington, Seattle, WA and* [3]*Biomedical & Pharmaceutical Sciences, University of Montana, Missoula, MT.*

Despite data linking asbestos exposure with production of autoantibodies, questions remain about the role of the autoantibodies in subsequent disease. Residents of Libby, Montana have experienced significant exposure to amphibole asbestos due to the mining of asbestos-contaminated vermiculite near the community over several decades. This population predominantly exhibits pleural disease, and an autoimmune-like disorder that has yet to be well defined. This study investigated the relationship between autoimmunity and asbestos related disease (ARD). Serum samples of subjects from Libby were evaluated for anti-nuclear antibodies (ANA), and for anti-mesothelial antibody (AMA) using cell based ELISA testing. In accord with previous studies, 62% of the Libby samples were ANA positive, a frequency much higher than expected for a typical healthy population. The odds of having pulmonary disease was nearly four times greater for individuals positive for ANA compared with individuals negative for ANA. The Libby population also exhibited a significantly higher frequency of AMA than the control group. A total of 23 Libby subjects had significantly high AMA levels and were established as AMA positive. Of these subjects nearly 75% (17 subjects) had pleural disease. Therefore, the odds of having pleural disease was 4.72 times greater for individuals with positive AMA compared to subjects without positive AMA. In contrast there was no significant relationship between AMA positivity and interstitial disease. This raises the possibility that AMAs are contributing to the ARD in these patients. Additional research is needed to further establish the relationship, but an association between AMA and clinical disease progression might lead to an excellent target for therapeutic intervention, or mechanisms to better define and monitor disease in this patient population.

PS 1510 INVOLVEMENT OF OXIDATIVE STRESS IN TRICHLOROETHENE-MEDIATED AUTOIMMUNITY: DOSE- AND TIME-RESPONSE STUDY.

G. Wang, J. Wang, X. Fan and M. Khan. *Pathology, University of Texas Medical Branch, Galveston, TX.*

Trichloroethene (TCE), a common environmental contaminant, is known to induce autoimmunity. Previous studies in our laboratory showed increased oxidative stress in TCE-mediated autoimmunity. To further establish the role of oxidative stress and to investigate the mechanisms of TCE-mediated autoimmunity, dose- and time- response studies were conducted in female MRL+/+ mice by treating them with TCE via drinking water at doses of 0.5, 1.0 or 2.0 mg/ml for 12, 24 or 36 weeks. TCE exposure led to dose-dependent increases in anti-malondialdehyde (MDA)- and anti-4-hydroxynonenal (HNE)-protein adduct antibodies in the sera at 24 and 36 weeks, but the increases were greater at 36 weeks. However, no changes in these antibodies were observed at 12 weeks. The increases in these antibodies were associated with significant elevation in serum anti-nuclear- and anti-ssDNA-antibodies at 24 weeks and, to a greater extent, at 36 weeks, suggesting an association between TCE-induced lipid peroxidation and induction of autoimmunity. Moreover, splenocytes from mice treated with TCE for 24 weeks secreted significantly higher levels of IL-17 and IL-21 than did splenocytes from controls following 72 h stimulation with MDA-mouse serum albumin (MDA-MSA) or HNE-MSA adducts, and the increased release of cytokines showed a dose-dependent response. Interestingly, the cytokine release was even greater in mice treated with TCE for 36 weeks. This study not only provides an evidence for the existence of a dose-and time-dependent association among TCE exposure, oxidative stress and induction of autoimmunity, but also suggests that MDA- and/or HNE-modified proteins act as an immunologic trigger and activate Th17 cells, thus contributing to an autoimmune response. Supported by NIH ES016302.

PS 1511 EFFECTS OF LIBBY AMPHIBOLE ASBESTOS EXPOSURE ON TWO RAT MODELS OF RHEUMATOID ARTHRITIS.

K. D. Salazar, C. B. Copeland and R. W. Luebke. *Cardiopulmonary and Immunotoxicology Branch, Environmental Public Health Division, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC.*

Epidemiological data suggest that occupational exposure to the amphibole-containing vermiculite in Libby, MT was associated with increased risk for developing autoimmune diseases and had an odds ratio of 3.23 for developing rheumatoid arthritis (RA). Our goal was to determine whether exposure to Libby amphibole (LA) modulates the disease course of RA. We utilized the collagen-induced arthritis

(CIA) and the Streptococcal cell wall (SCW) models of RA. The SCW arthritis model induces a local inflammatory reaction in one hind ankle that mimics the physiological milieu associated with RA in humans. The CIA model is a systemic model that produces paw inflammation in all 4 limbs and autoantibodies similar to human patients with RA. To determine if LA affects the onset and progression of RA, female Lewis rats were equally divided into 6 asbestos dose groups and 1 vehicle control group. Rats were intratracheally instilled biweekly for 13-weeks with total doses of 0.15, 0.5, 1.5, 5.0 mg LA or 0.5 or 1.5 mg amosite asbestos (positive control). Following the final instillation, joint inflammation was induced with either the SCW or CIA model. Swelling was quantified with a digital caliper and serum was analyzed for autoantibodies via ELISA and indirect immunofluorescence. Preliminary results from 2 experiments suggest that amosite, but not LA, exposure enhanced the magnitude of ankle swelling in the SCW model. However, neither LA nor amosite exposure affects the kinetics of the join inflammation in the CIA model. Both amosite and LA exposure increased the number of rats with circulating anti-nuclear antibodies (ANA). The majority of ANA-positive animals presented a similar ANA staining pattern which indicates the upregulation of autoantibodies with similar specificity. Future studies will identify the specificity of the upregulated ANA and characterize the effect of amphibole exposure on RA-associated antibodies. This abstract does not reflect EPA policy.

PS 1512 ESR SPIN-TRAPPING INVESTIGATION OF LUNG FREE RADICALS IN AN ANIMAL MODEL OF INTERSTITIAL PNEUMONIA ASSOCIATED WITH AUTOIMMUNE DISEASE.

M. B. Kadiiska, H. Miyakawa, J. Jiang and R. Mason. *NIEHS/NIH, Research Triangle Park, NC.*

The lung is frequently involved in autoimmune disease and complications involving the lung include mostly interstitial pneumonia (IP). However, the pathogenesis and mechanisms underlying the development of IP associated with autoimmune disease remain unknown. In this investigation, we studied the free radical generation involved in the development of IP in autoimmune disease murine model by using in vivo spin-trapping methodology. We observed an electron spin resonance (ESR) spectrum of the spin trap α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (POBN) radical adducts detected in the lipid extract of lungs in autoimmune-prone mice after intratracheal instillation of staphylococcal enterotoxin B (SEB). The POBN adducts detected by ESR were assigned as lipid-derived. Their presence was paralleled by infiltration of macrophages and neutrophils in the bronchoalveolar lavage fluid. To further investigate the mechanism of free radical generation, mice were pretreated with the macrophage toxicant gadolinium chloride (GdCl3), which significantly suppressed the free radical generation. Free radical generation was also decreased by pretreatment with the xanthine oxidase inhibitor allopurinol, the iron chelator Desferal, and the inducible nitric oxide synthase (iNOS) inhibitor 1400W. Histopathologically, these drugs significantly reduced both the cell infiltration to alveolar septal walls and the synthesis of pulmonary collagen fibers. Experiments with NADPH oxidase knockout mice showed that NADPH oxidase did not contribute to lipid radical generation. These results suggest that lipid-derived free radical production is essential in the manifestation of IP and that a macrophage toxicant, an XO inhibitor, an iron chelator, and an iNOS inhibitor protect against both radical generation and the manifestation of IP. Therefore macrophage toxicants, XO inhibitors, iron chelators or iNOS inhibitors may be potential therapeutic agents against alveolitis and fibrosis in treating IP.

PS 1513 B1A CELL ACTIVATION BY GLUTAMATE.

R. Marcum and J. C. Pfau. *Biological Sciences, Idaho State University, Pocatello, ID.*

Asbestos exposure has been implicated in an increased risk of autoimmunity. Direct exposure to asbestos has not been shown to activate B cells; however asbestos exposure does cause an increase of reactive oxygen species in macrophages. One mechanism by which macrophages may reduce the effects of these reactive oxygen species involves the xCT transporter, which is upregulated on macrophages after asbestos exposure. xCT is an amino acid transporter which imports cystine and exports glutamate. While the role of the imported cystine is likely for synthesis of cysteine-based antioxidants, the function of the exported glutamate in the immune system is not clear. We hypothesize that this exportation of glutamate is involved in the activation of B cells. In this study the presence of glutamate receptors on CH12.LX, a B1a cell line, was investigated by flow cytometry. The glutamate receptors mGluR5 and NMDA receptor were shown to be expressed on the cells. Additional work was done to investigate the effects on CH12.LX cells exposed to asbestos treated macrophage media as well as the effects of glutamate receptor agonist and antagonists. RAW 264.7 macrophages were exposed to asbestos for various time points, and the media was collected. The CH12.LX cells were then treated with media from untreated or asbestos exposed macrophages. Effects from the asbestos treated macrophage media, containing approximately 100μM glutamate, as measured by AmplexRed assay, on CD12.LX cells include a decrease in proliferation and increased production of IL-10. These effects were decreased by treatment with mGluR5 and NMDA receptor antagonists. These results suggest that glutamate is involved in the activation of B1a cells, making the xCT transporter a possible mechanism leading to autoimmune responses following asbestos exposure.

PS 1514 SEX HORMONES DETERMINE WHETHER IL-33 PROTECTS OR EXACERBATES AUTOIMMUNE HEART DISEASE.

M. Coronado, D. Bedja, A. Bucek, K. Gabrielson and D. Fairweather. *Johns Hopkins University, Baltimore, MD.*

Autoimmune myocarditis is an inflammatory heart disease that progresses to dilated cardiomyopathy (DCM) and heart failure and occurs at a higher incidence in men than women. In order to determine why disease is worse in men, we inoculated male and female BALB/c mice with 103 PFU of coxsackievirus B3 (CVB3) and cardiac myosin ip to induce autoimmunity. During acute myocarditis (day 10 post infection, pi), males developed more severe myocarditis than females that progressed to DCM by day 35pi. We showed previously that increased CVB3 myocarditis in males is associated with increased numbers of mast cells (MCs) and macrophages that express TLR4 and IL-1b. Interleukin-33 (IL-33) is a Th2 cytokine found to be cardioprotective in a mouse model of cardiac overload, where it reduced hypertrophy and improved survival. ST2 is the receptor for IL-33 that is expressed on MCs, macrophages and cardiac myocytes. Increased levels of soluble ST2 (sST2) in sera correlate with impaired LV function and heart failure. In contrast, administration of recombinant (r)IL-33 in mice increases inflammation in autoimmune models. Here, we found that IL-33 and ST2 levels in the heart were significantly lower in males compared to females at day 10. Gonadectomy (GDX) of males reduced inflammation while increasing IL-33 in the heart. This was reversed with testosterone (TE) replacement in GDX males. GDX males appeared immunologically like females, with reduced TLR4+IL-1b+ macrophages and increased IL-4 and regulatory T cells. Thus, we show that TE reduces cardioprotective IL-33 levels in the heart of males during acute myocarditis. However, when we inoculated male BALB/c mice with rIL-33 we found that males developed significantly increased acute myocarditis and sera sST2 levels compared to PBS controls, similar to results observed in other autoimmune models. We hypothesize that high IL-33 activates TLR4+IL-1b+ MCs and macrophages that express the ST2 receptor resulting in exacerbated disease in males. Thus, sex hormones determine whether IL-33 is protective or damaging to the heart following CVB3 infection.

PS 1515 EFFECT OF TISSUE TRANSGLUTAMINASE ANTIBODIES ON CELIAC DISEASE.

R. N. Ndeto and B. Fraij. *Biology, Chemistry, Environmental Health Science, Benedict College, Columbia, SC.* Sponsor: B. Cummings.

Celiac disease comprises intolerance against dietary gluten present in wheat, rye, and barley, and one of to the most common food related life long disorders. Now a day's celiac disease is conceived as an autoimmune mediated systemic disorder commonly presenting as enterophathy in genetically susceptible individuals. The most obvious features distinguishing celiac disease from other small intestinal enteropathies is the presence of auto antibodies against trans glutamines (TGC or TG2) during gluten containing diet. TGC is known to crosslink gliadin peptides to itself enabling the progression of celiac disease. The celiac disease-specific anti-TGC antibodies can modulate epithelial and neuronal cell as well as vascular biology. We studied if antibodies to tissue transglutaminase (TGC) might increase or decrease the enzymatic activities to TGC and there by enhance its cross linking function. It was reported that the conversion of TGC (80 kDa) to a smaller TG form (55 kDa) increases transglutaminase cross-linking activity. Incubation of lysates from RBC or MDA cells with celiac serum resulted in the conversion of the TGC to TG and increases in cross-linking activity when compared to normal serum, therefore, TGC antibodies might be the causative factor in the development of the extraintestinal symptoms occurring in celiac disease. Normal serum also resulted in the conversion of the TGC to TG and increases in cross-linking activity when compared to control with no serum, a condition that may be comparable to injury or wounds that lead to rapid enzymatic transamidation activation.

PS 1516 THE USE OF DMELS IN REACH RISK CHARACTERIZATION.

R. Roy, L. Milchak, N. Pechacek, J. Walton and R. Skoglund. *Medical Department, 3M Company, St. Paul, MN.*

Under the European Union's Registration, Evaluation, Authorization, and restriction of CHemicals (REACH) regulation, risk characterization (RC) for human health endpoints is done by comparing the ratio (called the Risk Characterization

Ratio; RCR) of the measured or modeled exposure level for a chemical to the health hazard information for the chemical. For threshold human health effect endpoints, the health hazard information is expressed as the Derived No-Effect Level (DNEL). For non-threshold health effects (e.g. genotoxic carcinogens, as per REACH) the health hazard information is expressed as the Derived Minimal Effect Level (DMEL). The European Chemicals Agency (ECHA) has developed guidance for the calculation of both DNELs and DMELs. For some chemicals, such as acrylamide, it is possible to calculate both a DNEL and DMEL. In cases like this, ECHA states that the lowest value (either the DNEL or DMEL) is used in the RC. In this exercise, both DNELs and DMELs were calculated, using ECHA guidance, for worker exposure to acrylamide via the long-term, inhalation exposure pathway. Toxicity data were obtained from the published literature. Worker DNELs ranged from 0.06 - 0.2 mg/m3 and DMELs ranged from 0.0001 - 0.0002 mg/m3. Using the DNELs and DMELs, RCRs were calculated (exposure concentrations for the numerator of the RCR were obtained from published occupational exposure studies). The impact of: 1) using chemical-specific oral absorption data for acrylamide (≥ 80%) vs a default value for the route-to-route extrapolation of the toxicity data; 2) the use of ECHA default values on the calculation of the DNELs and DMELs and 3) using the DMEL (vs DNEL), as recommended by ECHA, in the RCR is shown. The importance of an accurate, reliable exposure assessment as well as the implementation of exposure-reduction strategies (REACH Risk Management Measures; RMMs) like local exhaust ventilation and/or respirator use (which can provide 80 to ≥ 90% exposure reduction) to control risk to a low level of concern (i.e. from an initial RCR > 10, with no RMMs to a final RCR < 1, with application of RMMs) is shown.

PS 1517 THE ROLE OF ANNUAL VALIDATION IN RISK MANAGEMENT IN BIOSAFETY LEVEL 3 LABORATORIES.

D. Harbourt[1, 2], J. Glass[2, 3], J. Osorio-Sanchez[2, 3], T. J. Traum[2, 3] and D. Wilson[2, 3]. [1]*National Biosafety and Biocontainment Training Program, National Institutes of Health, Bethesda, MD,* [2]*Division of Occupational Health and Safety, National Institutes of Health, Bethesda, MD and* [3]*Office of Research Services, National Institutes of Health, Bethesda, MD.*

Since 2001, there has been an increase in the funding of high containment laboratories (HCLs) operating at Biosafety Level 3 (BSL-3). BSL-3 labs are designed to contain indigenous or exotic agents with the potential for aerosolized transmission resulting in serious infection. BSL-3 labs require specialized design and lab practices according to the Biosafety for Microbiological and Biomedical Laboratories 5th Ed (BMBL). The BMBL states validation of BSL-3 lab facilities and procedures is recommended but required if select agents are used. A major part involves testing the HVAC system to ensure that the lab remains under negative pressure during normal conditions and failure scenarios. Failure scenario testing was carried out by evaluating airflow at each doorway within the BSL-3 lab. Airflow was monitored progressively from the entrance in the building corridor to the anteroom and into the BSL-3 lab. The NIH requires that airflow be progressively more negative at each room leading from building corridor into the lab with a differential pressure of at least 0.05 inches of water (W.G.). Validated differential pressure monitors (DPMs) were used during all failure scenarios including emergency power transition, failure of both the supply and exhaust (EF) HVAC systems and restoration of lab systems. Testing under normal operation revealed an insufficient pressure differential (PD) of 0.02 W.G. between the lab and anteroom. PDs remained unacceptable (0.02 W.G.) during emergency power testing. Airflow reversals (ARs) which are unacceptable according to the BMBL were observed for up to 30s during EF failure. Due to insufficient negative pressure differentials and ARs during failure testing, the labs were shutdown pending significant upgrades in lab systems required to operate at BSL-3 and minimize risk to the lab staff working in and around the HCL.

PS 1518 ICCVAM EVALUATION AND RECOMMENDATIONS ON THE NON-RADIOACTIVE LLNA: BRDU-ELISA FOR EVALUATING ALLERGIC CONTACT DERMATITIS HAZARDS.

A. Jacobs[1], J. Matheson[2], M. Wind[3], V. Malshet[2], J. Toy[2] and W. Stokes[4]. [1]*U.S. FDA, Silver Spring, MD,* [2]*Consumer Product Safety Commission, Bethesda, MD,* [3]*Consumer Product Safety Commission, Bethesda, MD and* [4]*NICEATM, NIEHS, Research Triangle Park, NC.*

ICCVAM assessed the usefulness and limitations of the LLNA: BrdU-ELISA, a nonradioactive local lymph node assay (LLNA) that measures the amount of BrdU incorporation into the DNA of proliferating lymphocytes as an indicator of potential allergic contact dermatitis (ACD) hazards. Accuracy when compared to the traditional LLNA was assessed based on data generated with 43 substances and using several different stimulation indices (SI) as decision criteria. Optimal performance was achieved using SI ≥ 1.6; the LLNA: BrdU-ELISA correctly identified all 32 LLNA sensitizers (0% [0/32] false negatives) and 9/11 LLNA nonsensitizers (18% [2/11] false positives). The 2 false positives had maximum SI between 1.6-1.9. There were 18 substances with repeat tests; results for 85% (11/13) of the LLNA sensitizers and 60% (3/5) of the LLNA nonsensitizers were 100% concordant among the repeat LLNA: BrdU-ELISA tests. ICCVAM concluded that the accuracy and reproducibility of the LLNA: BrdU-ELISA support its use to identify potential skin sensitizers and nonsensitizers. ICCVAM recommends SI ≥ 1.6 to identify potential sensitizers since there were no false negatives relative to the LLNA. In testing situations where dose-response information is not required, or negative results are anticipated, ICCVAM recommends that the single-dose reduced LLNA: BrdU-ELISA should be considered and used, thereby reducing animal use by up to 40%. The ICCVAM-recommended protocol formed the basis of the recently adopted OECD Test Guideline 442B for the LLNA: BrdU-ELISA. Because the LLNA: BrdU-ELISA does not require radioactive reagents, more institutions can take advantage of the reduction and refinement benefits afforded by the LLNA compared to traditional guinea pig methods for ACD testing. The LLNA: BrdU-ELISA will also eliminate the environmental hazard associated with use and disposal of radioactive materials used in the LLNA.

PS 1519 ICCVAM EVALUATION OF THE LOCAL LYMPH NODE ASSAY (LLNA) FOR POTENCY CATEGORIZATION OF CHEMICALS CAUSING ALLERGIC CONTACT DERMATITIS IN HUMANS.

P. Brown[1], J. Matheson[2], A. Jacobs[1], T. McMahon[3], D. Germolec[4], M. Wind[5] and W. Stokes[6]. [1]*U.S. FDA, Silver Spring, MD,* [2]*Consumer Product Safety Commision, Bethesda, MD,* [3]*U.S. EPA, Washington, DC,* [4]*NIEHS, Research Triangle Park, NC,* [5]*Consumer Product Safety Commission, Bethesda, MD and* [6]*NICEATM, NIEHS, Research Triangle Park, NC.*

ICCVAM evaluated the LLNA as a stand-alone test method to determine potency categorization of chemicals that may cause allergic contact dermatitis (i.e., potential skin sensitizers). The dose per unit skin area that induces a 5% positive response rate (i.e., DSA05) in the human maximization test (HMT) or human repeat-insult patch test (HRIPT) was used as the human induction threshold. Substances with induction thresholds ≤500 μg/cm2 were classified as "strong" human sensitizers. The extent to which the LLNA EC3 (estimated concentration needed to produce a stimulation index of 3, the threshold value for a positive response) correctly categorizes strong human sensitizers was evaluated by examining 136 substances with both LLNA and human data. Using EC3 ≤ 2%, a criterion recently adopted by the United Nations Globally Harmonized System of Classification and Labelling of Chemicals, correctly categorized 52% (14/27) of the strong human sensitizers. However, nearly half (48% [13/27]) of the strong human sensitizers had an EC3 > 2% (11/27) or were negative in the LLNA (2/27). Of the 11 strong human sensitizers with an EC3 > 2%, 91% (10/11) had an LLNA EC3 value between 2% and 10%. ICCVAM concludes that the LLNA can be used to categorize substances as strong sensitizers when EC3 ≤ 2% but cannot be used as a stand-alone assay to predict sensitization potency categories. Substances producing an LLNA EC3 between 2% and 10% will require additional information to determine that the substance should not be categorized as a strong sensitizer. To improve the accuracy of the LLNA for identifying strong sensitizers, ICCVAM encourages the development and evaluation of integrated decision strategies that consider other types of relevant information such as quantitative structure-activity relationships, structural alerts, peptide reactivity, in vitro data, human data or experience, and existing data from similar chemical entities.

PS 1520 ICCVAM EVALUATION AND RECOMMENDATIONS ON THE NONRADIOACTIVE LLNA: DA FOR EVALUATING ALLERGIC CONTACT DERMATITIS HAZARDS.

J. Mathesono[1], A. Jacobs[2], M. Wind[3], P. Brown[4], R. Ward[4], E. Margosches[4] and W. Stokes[5]. [1]*Consumer Product Safety Commission, Bethesda, MD,* [2]*U.S. FDA, Silver Spring, MD,* [3]*Consumer Product Safety Commission, Bethesda, MD,* [4]*U.S. EPA, Washington, DC and* [5]*NICEATM, NIEHS, Research Triangle Park, NC.*

ICCVAM assessed the usefulness and limitations of the LLNA: DA, a nonradioactive local lymph node assay (LLNA) that measures ATP content as an indicator of lymphocyte proliferation and, in turn, potential allergic contact dermatitis (ACD) hazards. Accuracy when compared to the traditional LLNA was assessed based on data generated with 44 substances and using several different stimulation indices (SI) as decision criteria. Optimal performance was achieved using SI ≥ 1.8; the LLNA: DA correctly identified all 32 LLNA sensitizers (0% [0/32] false negatives), and 9/12 LLNA nonsensitizers (25% [3/12] false positives). The 3 false positives had maximum SI between 1.8-2.5. There were 14 substances with repeat tests; results for 80% (8/10) of the LLNA sensitizers and 75% (3/4) of the LLNA nonsen-

sitizers were 100% concordant among the repeat LLNA: DA tests. ICCVAM concludes that accuracy and reproducibility of the LLNA: DA support its use to identify potential skin sensitizers and nonsensitizers. ICCVAM recommends SI ≥ 1.8 to identify ACD hazards since there were no false negatives relative to the LLNA. In testing situations where dose-response information is not required, or negative results are anticipated, ICCVAM recommends that the single-dose reduced LLNA: DA should be considered and used, thereby reducing animal use by up to 40%. The ICCVAM-recommended protocol formed the basis for the recently adopted OECD Test Guideline 442A. Because the LLNA: DA does not require radioactive reagents more institutions can take advantage of the reduction and refinement benefits afforded by the LLNA compared to traditional guinea pig methods for ACD testing. The LLNA: DA will also eliminate the environmental hazard associated with use and disposal of radioactive materials used in the LLNA.

PS 1521 USING FEWER ANIMALS TO IDENTIFY CHEMICAL EYE HAZARDS: REVISED CLASSIFICATION CRITERIA NECESSARY TO MAINTAIN EQUIVALENT HAZARD LABELING.

W. Stokes[1], J. Haseman[2], E. Lipscomb[3], J. Truax[3], N. Johnson[3], B. Jones[3] and D. Allen[3]. *[1]NICEATM, NIEHS, Research Triangle Park, NC, [2]Consultant, Raleigh, NC and [3]ILS, Inc., Research Triangle Park, NC .*

U.S. Federal Hazardous Substances Act (FHSA) regulations specify eye safety testing procedures and hazard classification criteria for chemicals and products regulated by CPSC and OSHA. Current regulations require up to three sequential tests of six animals per test, with decisions on the need for subsequent tests based on the number of positive responses observed. Testing conducted in accordance with the OECD test guideline for eye irritation and corrosion can also be used, but current FHSA regulations do not provide criteria to classify results from this 3-animal test. Therefore, an analysis was conducted to determine classification criteria for results from a 3-animal test that would provide hazard labeling equivalent to that provided by current FHSA regulations. This analysis compared the frequency at which the current FHSA classification criteria would identify substances as ocular irritants with the frequency at which a classification criterion of either one or two positive animals out of three would identify these substances. The resulting classifications that would be assigned by each of the three criteria were also compared using four different underlying response rates (20, 40, 50, and 75%). For these response rates, current FHSA requirements would identify 20, 73, 88, and >99% of substances as irritants, respectively, while using a criterion of at least one out of three positive animals in a 3-animal test would identify 49, 78, 88, and 98% of substances as irritants, respectively. In contrast, using a criterion of at least two out of three positive animals in a 3-animal test would identify far fewer irritants, with detection rates of 10, 35, 50, and 84%, respectively. We conclude that using classification criteria of one or more positive animals in a 3-animal test will provide the same or greater level of eye hazard labeling as current FHSA requirements, while using up to 83% fewer animals. ILS Staff supported by NIEHS contract N01-ES-35504.

PS 1522 FROM HAZARD-BASED TO TISSUE DOSE-BASED TESTING—REDUCTION AND REFINEMENT.

J. Bessems. *National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.*

Research into apparently clashing paradigms in current chemical safety testing led to unorthodox opportunities to replace, reduce and/or refine obligatory animal testing. In order to prevent human exposure to hazardous levels of chemicals, hazardous levels in animals have to be established. Effects assessment to establish a point of departure comes upfront in risk assessment and is targeted at finding a hazard. Without a LOAEL, a NOAEL can not be established. Unfortunately, replacement of bioassays for systemic effects is a long-lasting challenge. Focusing on reduction and refinement might be more successful. In risk assessment, human exposure is measured, estimated or predicted completely independent from toxicity testing. Finally, the point of departure and exposure are compared, either by taking into account various assessment factors or via a margin of safety approach. This contribution provides an outline how increased focus on exposure assessment including assessment of target tissue dose by taking toxicokinetics into account can result in reduction and refinement using tissue dose-informed testing. Predicting tissue doses (using physiologically-based modeling) and establishing in vitro effect concentrations should come upfront. Comparing resulting figures may lead to significant reductions by waiving obligatory bioassays (tissue dose far below in vitro effect concentrations). It would leave only the need for some confirmatory testing at much lower tissue dose levels which will cause significantly less animal stress (refinement). Finally, reductions by focused testing can be achieved in more traditional paradigms by upfront in silico, in vitro and/or in vivo (screening) studies with respect to absorption, distribution, metabolism and excretion. Taking apparent specificities across species, across doses/exposures, across age and sex and across routes of exposure into account in planning effects bioassays will prevent testing in the wrong species, irrelevantly high doses, wrong age, sex, or exposure route.

PS 1523 PRACTICAL IMPLEMENTATION OF THE THRESHOLD OF CONCERN AND NOAEL-TO-LC50 RATIO FACTOR APPROACH TO DETERMINE ACUTE EFFECTS SCREENING LEVELS.

T. D. Phillips, S. E. Ethridge and R. L. Grant. *Toxicology Division, Texas Commission on Environmental Quality, Austin, TX.*

Effects Screening Levels (ESLs) are chemical-specific air concentrations set to protect human health and welfare and are used in the Texas air permitting process to assess the protectiveness of emission rate limits. Short-term ESLs are developed to evaluate acute intermittent exposures of 1 hour (h). The Texas Health and Safety Code requires the development of ESLs for as many air contaminants as possible, even chemicals with limited toxicity data (LTD). Grant et al. 2007 used a statistical analysis of a compiled database of LC50 and NOAEL inhalation data to establish two different methods for determining acute generic ESLs for LTD chemicals: Threshold of Concern (TOC) and NOAEL-to-LC50 (N-L) Ratio. In the TOC approach, acute inhalation lethality data are used to classify chemicals in different acute toxicity categories based on the GHS of Classification and Labeling of Chemicals proposed by the UN. Different health-protective concentrations are used for chemicals assigned to different GHS categories. For the N-L ratio approach, a 4h duration LC50 value is multiplied by a factor of 8.3E-5 to estimate health-protective air concentrations. These generic ESLs are used until adequate toxicity data are available. The Texas Commission on Environmental Quality (TCEQ) incorporated these approaches into the peer-reviewed Guidelines to Develop Effects Screening Levels, Reference Values, and Unit Risk Factors in 2006. TCEQ has implemented these approaches to determine generic ESLs for pentene isomers and hexane. These approaches have also been used to ground truth acute ESLs for the following chemicals derived when multiple-day studies were used (i.e., acceptable toxicity studies less than 24h were not available): 1,3-butadiene, 1-butene, 2-butene, isobutene, TexanolTM, toluene, tetrachloroethylene, ethylbenzene, and benzene. Generally, the TOC method was slightly more conservative than the N-L ratio approach. A weight-of-evidence approach is used to determine which method is more scientifically defensible.

PS 1524 REPEATED-DOSE PRECLINICAL TOXICITY STUDIES: HIGHEST DOSE TESTED=NOAEL—WHAT TO DO?

R. Garg, W. Bracken, D. Bury and K. Marsh. *Abbott Laboratories, Abbott Park, IL.*

Absence of Dose-Limiting Toxicity (DLT) in a repeat-dose toxicity study may raise questions regarding validity of the study, e.g. a) evidence if the highest dose was the maximum feasible dose or one that produces maximum feasible exposure, b) availability of data on alternative formulations or dosing regimens attempted to improve drug exposure and c) relation of achieved drug exposure at NOAEL to the predicted clinically efficacious exposure. A pre-IND stage Abbott compound (ABT-000) was generally well tolerated in animal toxicity studies. The highest dose in both the 4-week rat and dog toxicity studies was concluded as the NOAEL (180 and 50 mg/kg/day for rat and dog, respectively). In anticipation of questions from the regulatory agency and given the limited solubility and bioavailability of the drug substance, several preclinical formulations were evaluated to optimize the systemic exposure to ABT-000. From the test formulations, a vehicle containing Cremophore EL and vinylpyrrolidone dimer (VP-dimer) at 80:20 ratio was selected since it produced the highest systemic exposure to the drug. In both, rat and dog, the systemic exposure for ABT-000 was not dose proportional and declined at the highest dose tested. In view of this observation, the dose with highest exposure was selected for the rat and dog toxicity studies. As VP-dimer is not a common vehicle, a 4-week toxicity study on this vehicle component was also performed and included in the submission. As adverse effects related to VP-dimer could not be excluded, a saline control group was added to both the rat and dog toxicity studies. Given that the systemic exposure at the NOAEL was 83 to 87-fold higher than the predicted human efficacious exposure and supporting formulation data was available, the studies were accepted for the IND. In conclusion, even when the highest dose in a preclinical toxicity study reveals to be a NOAEL, this can be acceptable to a regulatory agency, when appropriate supporting data are provided and a good safety margin is evidenced

PS 1525 SAFETY ASSESSMENT OF FOOD AND FEED FROM BIOTECHNOLOGY-DERIVED CROPS WITH MODIFICATIONS THAT REGULATE ENDOGENOUS GENE EXPRESSION.

J. S. Petrick[1], W. Parrott[2], B. Chassy[3], J. Ligon[4], L. Meyer[5], J. Zhou[6], R. Herman[7], B. Delaney[8] and M. Levine[9]. *[1]Product Safety Center, Monsanto Company, Saint Louis, MO, [2]Crop and Soil Sciences, University of Georgia, Athens, GA, [3]College of ACES, University of Illinois, Urbana, IL, [4]Bayer CropScience, Research Triangle Park, NC, [5]Syngenta Biotechnology, Inc., Research Triangle Park, NC, [6]Johnson & Johnson, Raritan, NJ, [7]Dow AgroSciences, Indianapolis, IN, [8]Pioneer, A DuPont Company, Johnston, IA and [9]International Life Sciences Institute, Washington, DC.*

Agricultural biotechnology traits in development include those using tools such as transcription factors and RNA interference (RNAi) to modulate endogenous plant gene expression for the improvement of yields, tolerance to biotic and abiotic stresses, and nutritional quality. Biotechnology-derived crops are presently evaluated for food and feed safety relative to a conventional comparator according to an internationally-accepted safety assessment paradigm. This robust and comprehensive approach incorporates basic concepts from food safety, toxicology, nutrition, molecular biology, and plant breeding and has been used effectively by scientists and regulatory agencies for nearly 15 years. The main aspects of the comparative safety assessment process are reviewed and recommendations are provided for scientifically sound principles to evaluate the safety of biotechnology-derived crops developed using biological approaches that modify endogenous plant gene expression. Key considerations for applicability of the existing safety assessment process include: 1) The history of safe consumption of RNAi- mediating small RNAs (e.g., miRNAs) and regulatory proteins such as transcription factors; 2) The growing scientific literature describing the central role that RNAi and transcription factors have played in plant domestication and conventional breeding; 3) Crops engineered using RNAi constructs are not expected to produce heterologous proteins; 4) Modulation of plant gene expression may result in quantitative differences in levels of endogenous plant components (but not de novo components) that can be assessed through compositional analysis.

PS 1526 USE OF CLUSTER ANALYSIS IN PESTICIDE INERT INGREDIENT RISK ASSESSMENT.

A. S. Duggan[1], J. Johnston[2], J. Messina[2], K. Akkari[3], D. Hillebold[4], G. Lindner[5] and P. McCain[6]. *[1]Health Practice Center for Toxicology and Mechanistic Biology, Exponent, Inc., Philadelphia, PA, [2]Health Practice Center for Chemical Regulation and Food Safety, Exponent, Inc., Washington, DC, [3]APD/RD, BASF Corp., Research Triangle Park, NC, [4]Surface Chemistry, Akzo Nobel, LLC, Chicago, IL, [5]Crop Care Applications, Croda, Inc., New Castle, DE and [6]Regulatory Affairs, Syngenta Crop Protection, Inc., Greensboro, NC.*

The poster illustrates the use of read-across methodology and dietary risk assessment for oleochemical surfactants that are used as inert ingredients in pesticide formulations. The August 9, 2006, revocation of 130 food tolerance exemptions resulted in unprecedented challenges for EPA and industry (pesticide registrants and surfactant manufacturers) to evaluate reproductive and developmental toxicity for more than 20 classes of inert ingredients within three years. Structurally similar compounds have similar physical, metabolic and toxicological properties. It is possible to bridge (read-across) results from one compound to others within a defined cluster of chemically-related compounds. EPA and the OECD applied cluster analysis in the High Production Volume (HPV) Programs to reduce the number of toxicity tests, laboratory animals and the time required to review the study data. The same methodology was successfully used to conduct OECD 422 Studies (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Tests) data development plans. The OECD 422 test results were used in conjunction with the EPA screening-level dietary exposure model for inert ingredients (I-DEEM) to assess dietary exposure and risks for the US population, adults and children. The methodology was validated and resulted in the successful reassessment and retention of the food use tolerance exemptions of the inert ingredients.

PS 1527 OPTIONS FOR INCREASED REGULATORY OVERSIGHT OF COSMETICS IN THE U.S.

N. Beck[1], K. Sullivan[1] and C. Willet[2]. *[1]Physicians Committee for Responsible Medicine, Washington, DC and [2]People for the Ethical Treatment of Animals, Norfolk, VA.*

Cosmetics have recently become one of the focal points of efforts to strengthen chemical regulations in the US. Under the current law, cosmetics manufacturers are responsible for substantiating the safety of their products, but for most ingredients, specific toxicity testing is not required, nor is pre-market approval from the Food and Drug Administration (FDA). Concern over the long-term safety of cosmetics has prompted legislative efforts to increase oversight. Emerging regulatory models in the US were analyzed, namely the "FDA Globalization Act of 2009" and the "Safe Cosmetics Act of 2010." Specifically, each bill's approach to hazard and risk assessment, was examined, including their flexibility in information requirements and potential for incorporating evolving science. Then, current US cosmetics regulations were compared with international approaches, revealing broad agreement overall. Finally, the proposed US models were addressed in the context of the EU's distinctive position on animal testing. EU law gradually phases out the use of animals for cosmetics testing, with a complete ban on the marketing of animal-tested products by 2013. The market ban presents significant implications for trade with the EU, which should be considered when drafting new legislation in the US. The testing and marketing prohibitions in the EU have also impacted the state of regulatory toxicology, with the impending ban serving as a tool to drive progress in the development of in vitro and in silico test methods. In order to efficiently assess cosmetic ingredients and advance international harmonization, proposals to reform US cosmetics regulations should seek to facilitate the development of modern, harmonized approaches to testing and assessment.

PS 1528 THE SHE CELL TRANSFORMATION ASSAY—ITS USE IN HAZARD AND RISK ASSESSMENT FOR REACH.

A. H. Poth. *Genetic and Alternative Toxicology, Harlan Cytotest Cell Research GmbH, Rossdorf, Germany.*

The 2-year bioassay is the standard method for carcinogen detection which is time and resource intensive. Many short-term test, especially genotoxicity tests have been developed to aid in identification of potential carcinogens. However, the endpoint of theses systems is genotoxicity and their concordance between rodent bioassays is only about 60% and a battery of short-term genotoxicity tests can not improve the overall concordance. In vitro cell transformation tests using SHE- and Balb/c3T3-cells simulate the process of animal two-stage carcinogenesis. These tests are suited for the in vitro detection of a carcinogenic potential of test compounds in safety and risk assessment. Results from cell transformation assays can provide information, which in combination with data from other testing methods, are useful for identifying the carcinogenic potential of chemical compounds. The results of these assays in combination with other information such as genotoxicity data, structure activity analysis, in vivo toxicity data and pharmaco-/toxicokinetic information can facilitate a relatively comprehensive assessment of a carcinogenic potential of a chemical. For prediction of the carcinogenic potential of compound the SHE cell transformation assay is included in the endpoint specific guidance document for REACH. In the first phase of REACH (substances > 1000 tonnage per year), data of the SHE cell transformation were included in the hazard and risk assessment. Case studies are provided for heavy metal powders and alloys, where the SHE cell transformation data were used for mechanistic investigations beside other in vitro test systems. SHE cell transformation data are also provided of a chemical class (aromatic amines) where genotoxicity data do have only limited value and for a compound with an structural alert for carcinogenicity (pararosaniline derivatives), where SHE cell transformation data were used to demonstrate the similarities across a chemical category.

PS 1529 DEVELOPMENT OF A HEALTH RISK-BASED SURFACE CONTAMINATION CLEAN-UP STANDARD FOR OCCUPATIONAL EXPOSURE TO BERYLLIUM.

P. Damian. *SCS Engineers, West Sacramento, CA.*

A health risk-based surface contamination cleanup standard (SCS) for beryllium (BE) was developed to facilitate the safe transfer of property (equipment and buildings) previously used in BE-related processes. Previous SCSs for BE were primarily based on Department of Energy (DOE) housekeeping criteria rather than health risks. Quantitative health risk assessment methods were used to develop an occupational SCS that explicitly considers the relevant exposure pathways and toxicity endpoints, including both cancer and non-cancer endpoints. For the cancer endpoint at the 1E-06 risk level, the analysis resulted in an SCS of 17 $\mu g/100\ cm^2$ based on resuspension of settled dust and subsequent inhalation exposure only (BE is regulated as a carcinogen by the inhalation route only). For the non-cancer endpoint, the analysis resulted in an SCS of 0.07 $\mu g/100\ cm^2$ based on dermal absorption, incidental ingestion following dermal contact, and inhalation. The non-cancer SCS was determined virtually entirely by the dermal absorption exposure pathway, with negligible contributions from the incidental ingestion and inhalation pathways. This analysis shows that application of the non-cancer SCS in BE monitoring and control programs will adequately protect workers from both the cancer and non-cancer health effects of BE when surface contamination is the primary source of BE exposure.

PS 1530 MINIMAL RISK LEVELS FOR STYRENE.

S. Chou[1] and L. Ingerman[2]. [1]*Division Toxicology and Env.Med. ., ATSDR/CDC, Atlanta, GA and* [2]*SRC, Inc., Syracuse, NY.* Sponsor: B. Fowler.

Styrene is a high production chemical. Manufactured styrene is primarily used in the production of polystyrene plastics, resins and copolymers. In the general population, exposure to styrene occurs primarily by inhalation of contaminated indoor air and can be attributed to emissions from building materials, consumer products and tobacco smoke. The workplace or home office may have substantially higher levels of airborne styrene, due to emissions from laser printers and photocopiers. Exposure to styrene may also occur through ingestion of contaminated food or water. The inhalation and oral database provides evidence that the nervous system is the most sensitive target following acute and chronic duration exposures to styrene. After reviewing the literature, an MRL of 5 ppm was derived for acute-duration inhalation exposure to styrene based on no-observed-adverse-effect level (NOAEL) for alterations in tests of reaction time, memory, attention, color discrimination and olfactory threshold in humans (Ska et al. 2003) and an MRL of 0.2 ppm was derived for chronic-duration inhalation exposure based on lowest-observed-adverse-effect level (LOAEL) for neurological effects from a meta-analysis of occupational exposure studies (Benignus et al. 2005). In addition, an MRL of 0.1 mg/kg/day for styrene was derived for acute-duration oral exposure based on for impaired learning in rats (Husain et al. 1985). These MRLs are presented and discussed in the ATSDR toxicological profile for styrene released in September 2010. These substance-specific health guidance values are intended to serve as screening levels for use by health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

PS 1531 RISK ASSESSMENT FOR ETHYL *TERTIARY*-BUTYL ETHER (ETBE) TO DETERMINE ACCEPTABLE DRINKING WATER LEVELS.

V. S. Bhat, G. L. Ball and C. J. McLellan. *NSF International, Ann Arbor, MI.*

ETBE can be detected in the extract water of some cross-linked polyethylene pipes that convey potable water. Human oral data for ETBE were not identified. In the key study, chronic drinking water exposure was associated with increased kidney weights and exacerbated chronic progressive nephropathy (CPN) severity in male and female F344 rats. The rats appeared to compensate for the reduced palatability of ETBE, so the results were considered reliable for interpretation. Available data support that the liver effects after gavage or inhalation exposure are mediated by adaptive mechanisms to metabolizing high or bolus ETBE doses as they were not observed after drinking water exposure. There are insufficient data to establish a mode of action other than α-2μ-globulin nephropathy in male rats. Interim sacrifices were not included to allow for histological assessment of kidney tubules in a less marked CPN stage. Recognizing the challenge in interpreting chemical-specific renal toxicity in rats with advanced CPN and α-2μ-globulin nephropathy, the point of departure for the Reference Dose (RfD) was the $BMDL_{10}$ of 220 mg/kg-day for exacerbated CPN severity in female rats. Exacerbated CPN severity was more amenable to benchmark dose modeling thus providing more confidence for risk assessment compared to kidney weight data. The dose metric was the received dose due to insufficient kinetic data to construct of model describing ETBE or its metabolites in blood, urine or target tissue for use as an internal dose metric. No tumor incidences were statistically increased in the key study and ETBE has low genotoxic potential. Using U.S. EPA (2005) guidelines, there is *inadequate information to assess carcinogenic potential* of ETBE due to the lack of chronic data in a second species. Gavage exposure during pregnancy was associated with maternal toxicity with no specific effects on reproduction or development. A 300-fold uncertainty factor was applied to the $BMDL_{10}$ to obtain a RfD of 0.7 mg/kg-day. This corresponds to a Total Allowable Concentration of 5 mg/L in drinking water assuming a 70 kg adult drinks 2 L/day.

PS 1532 HEALTH RISK ASSESSMENT FOR HYDROGEN PEROXIDE TO DETERMINE ACCEPTABLE DRINKING WATER LEVELS.

G. L. Ball, J. C. English and C. J. McLellan. *NSF International, Ann Arbor, MI.*

Acceptable levels for hydrogen peroxide (H_2O_2) in drinking water were determined due to its use as a drinking water disinfectant in combination with a small amount of silver. H_2O_2 participates in the maintenance of cellular homeostasis at endogenous, physiologic levels, and low exposure concentrations can induce the adaptive up-regulation of antioxidant enzymes. At progressively higher levels, a pro-inflammatory response is provoked and oxidative stress ensues. Local irritation occurs with time and concentration dependence, and catalase, among other enzymes, participates in the removal of H_2O_2 from tissue. The critical effects observed in laboratory animals included dose-dependent increases in glandular stomach erosion, duodenal hyperplasia, and duodenal carcinoma in mice given H_2O_2 chronically in drinking water. The lesions correlated inversely with catalase activity and were attributed to chemical irritation. H_2O_2 *in vitro* induced forward and reverse mutations, chromosomal aberrations, DNA damage/repair, and sister chromatid exchanges. The lack of genotoxicity *in vivo* is likely related to detoxifying enzyme activities and endogenous radical scavenging substances. Although H_2O_2 produced duodenal carcinomas upon repeated administration in drinking water to catalase-deficient mice, these tumors failed to metastasize and hyperplasia readily resolved upon cessation of treatment. The data support the classification of *suggestive evidence of carcinogenic potential*, based on U.S. EPA (2005) guidelines for carcinogen risk assessment. The data additionally support a low-dose nonlinear mode of action and H_2O_2 was evaluated accordingly. A 100-fold uncertainty factor was applied to the $BMDL_{05}$ of 49 mg/kg-day for duodenal hyperplasia from a subchronic drinking water study in catalase-deficient mice to obtain a Reference Dose (RfD) of 0.5 mg/kg-day. The RfD corresponds to a Total Allowable Concentration of 8 mg/L in drinking water assuming a 70 kg adult drinks 2 L/day, taking into account the fact that the major known non-drinking water sources of ingested H_2O_2 constitute approximately 50% of the oral RfD.

PS 1533 LEAD SOIL SCREENING LEVELS AND CLEAN-UP AT CALIFORNIA HAZARDOUS WASTE SITES.

T. Behrsing[1], J. Carlisle[2], E. Sciullo[1], J. Spearow[1], B. Davis[1], K. Day[1] and M. Wade[1]. [1]*Department of Toxic Substances Control (DTSC), CalEPA, Sacramento, CA and* [2]*Office of Environmental Health Hazard Assessment (OEHHA), CalEPA, Sacramento, CA.*

In 2007, CalEPA developed a new toxicity evaluation of lead replacing the 10 μg/dL threshold blood lead concentration (PbB) with a source-specific "benchmark change" of 1 μg/dL (the estimated incremental increase in children's PbB reducing IQ by 1 point). Here we show the resulting derivation of the revised California Human Health Screening Levels (CHHSLs) using the new PbB criterion and newer blood lead population data. The updated CHHSLs of 80 and 320 mg/kg lead in soil for residential and industrial/commercial land use scenarios, respectively, are lower than previous Cal-modified USEPA Region 9 Preliminary Remediation Goals of 150 and 800 mg/kg. To evaluate lead risk and cleanup options, DTSC recommends calculating the 95 percent upper confidence limit on the arithmetic mean (95%UCL) lead concentration. If individual samples exceed the CHHSL, the exposure area itself would not exceed the CHHSL as long as the 95%UCL is below the CHHSL (assuming no hot spots). If data are insufficient to calculate a 95%UCL, comparison of the maximum detected concentration to the CHHSLs is appropriate. In such cases, additional sampling or cleanup may be warranted. In our experience, the upper lead concentration associated with a 95%UCL of 80 mg/kg is about 150 mg/kg unless there are hot spots. At sites A and B (with maximum lead concentrations of 2078 and 4100 mg/kg) removing samples with lead above 144 and 180 mg/kg lead, respectively, results in predicted 95%UCLs of ~80 mg/kg. The actual maximum threshold for lead to achieve a specific 95%UCL for a given site depends on many factors such as contaminant distribution and confirmation sampling results. Depending on site-specific conditions, cleanup goals consistent with a 95%UCL equal to the revised residential CHHSL can be approached by excavating contaminated soils with lead concentrations above approximately twice the revised CHHSL. A 95%UCL should be calculated using remaining soil and confirmation sampling data following remediation.

PS 1534 MYCOTOXINS: U.S., CANADIAN, EUROPEAN, AND JECFA REGULATORY STRATEGIES.

S. H. Henry. *Center for Food Safety and Applied Nutrition, U.S. FDA, Greenbelt, MD.*

U.S., Canadian, European, and JECFA (Joint World Health Organization/Food and Agriculture Organization Expert Committee on Food Additives) approaches to safety/risk assessment and regulatory guidance for mycotoxins of concern for human health, including aflatoxins and ochratoxin, will be summarized and compared. Aflatoxins are found in human and amimal foods, such as peanuts,corn and tree nuts as a result of fungal contamination during growth and after harvest. The U.S. Food and Drug Administration in its risk asesment of aflatoxin attempted to separate the risk of liver cancer attributed to aflatoxin from the risk from other factors, such as hepatitis B and C. This risk-based approach will be contrasted to an "as low as reasonably achievable" (ALARA) or European approach to aflatoxin standards. The conclusions of the JECFA regarding a safety/risk assessment of the potent liver carcinogen aflatoxin will be discussed with reference to the Codex Alimentarius Commission standards for aflatoxins in tree nuts (almonds, hazelnuts and pistachios) intended for further processing and ready-to-eat trade categories.

Next the approach of Health Canada to managing risk of the potent renal carcinogen ochratoxin A (OTA) in a market-based economy will be discussed. Ochratoxin occurs naturally in many foods, such as cereal-derived staples as well as other food commodities such as grapes, raisins, wine and coffee. Health Canada re-evaluated the tolerable daily intake (TDI), re-evaluated the European TDI and conducted a probabilistic risk assessment.

PS 1535 GENERATION OF ELECTRICAL POWER WITH WOODY BIOMASS: A SITE-SPECIFIC TECHNICAL EVALUATION OF EMISSIONS AND POTENTIAL HEALTH RISKS.

C. Teaf[1, 5], T. Davis[2], J. Doolittle[2], G. Morris[3], J. Levine[4] and D. Covert[5]. *[1]Florida State University, Tallahassee, FL, [2]ECT, Inc., Gainesville, FL, [3]Green Power Institute, Berkeley, CA, [4]American Renewables, LLC, Boston, MA and [5]Hazardous Substance & Waste Management Research, Tallahassee, FL.*

The generation of electric power on a commercial scale using woody biomass is receiving attention and achieving broader application in some areas of the US. As with any combustion facility, technical evaluations regarding potential human health risks or ecological impacts from a biomass-fired power plant must include an analysis of the specific characteristics of the proposed facility, including plant size, fuel quantities and types, boiler engineering, emissions control equipment, and applicable state or federal regulatory limits. A detailed preconstruction emission and risk evaluation of these and other factors was conducted concerning a woody biomass-fired facility that was undergoing regulatory review in Northcentral Florida. Projected emissions were modeled according to standard USEPA approaches (e.g., AERMOD) and impacts at all locations were shown to be well below all applicable air quality standards and guidelines. An evaluation of potential deposition rates and environmental impacts for specific Hazardous Air Pollutants (HAPs; e.g., dioxins/furans, mercury) was conducted according to USEPA protocols. Environmental concentrations of modeled HAPs showed that potential risks at all locations were well below acceptable limits, based upon comparisons with relevant standards and guidelines for air, soil, water, sediments and edible fish tissue.

PS 1536 EMERGENCY DO NOT CONSUME/DO NOT USE PUBLIC NOTIFICATIONS FOR DIRECT DRINKING WATER ADDITIVES.

C. J. McLellan, C. C. Willhite, V. S. Bhat and G. L. Ball. *NSF International, Ann Arbor, MI.*

Regulatory authorities and water purveyors are becoming increasingly concerned with accidental or intentional adulteration of municipal drinking water supplies. As a consequence, there is a need for short-term (acute) acceptable oral, topical, and ocular exposure levels to be used in emergency situations to notify the public. These levels, designated as Specific Concentration Levels (SCLs), have been derived for a number of direct drinking water additives using an approach analogous to the U.S EPA Reference Dose (RfD) and Health Advisory approaches. The SCLs were used to calculate emergency "Do Not Consume" levels based on the default 10 kg body weight and 1 L/day water consumption of a child, which is consistent with methods used to calculate one-day Health Advisories. As examples, a Do Not Consume level of 600 mg/L was derived for blended phosphates based on acute diarrhea after ingestion of a single oral dose of mono- and dibasic monophosphates. Based on mild irritation after topical application of 0.2% sodium metaphosphate to intact skin of sensitive volunteers, a Do Not Use limit of 8000 mg PO_4/L was assigned. Given the eye irritation seen in rabbits after direct instillation of 0.2% sodium metaphosphate, an acute ocular contact limit of 60 mg PO_4/L served as the overall Do Not Use level. These Do Not Consume and ocular or skin Do Not Use concentrations for phosphates also apply to acute exposures to zinc orthophosphate. A Do Not Consume concentration of 7 mg/L was derived for zinc from zinc orthophosphate based on clinical reports of gastrointestinal distress. While there were no empirical data upon which to derive a Do Not Use advisory for ocular or skin contact to zinc from zinc orthophosphate, potential irritation associated with the orthophosphate moiety is considered to be the governing factor for the ocular or topical use limits. To utilize the values derived here, the risk manager must take into account the exposure scenario and the pH of the water as well as the public health and safety consequences associated with a lack of water.

PS 1537 ENDPOINT SENSITIVITY IN FISH ENDOCRINE DISRUPTION ASSAYS: REGULATORY IMPLICATIONS.

Z. Dang. *RIVM, Bilthoven, Netherlands.* Sponsor: J. Bessems.

Identifying potential endocrine disrupting chemicals (EDCs) needs screening for elucidating mode of action (MOA) evidenced by biomarker endpoints and testing for intrinsic toxicological properties showed by apical endpoints. Risk assessment, however, is mainly based on apical endpoints but not MOA endpoints. Fish 21-day assay (e.g. OECD TG229) is considered as a screen. But this assay includes both biomarker and apical endpoints. This study explores the utility of results of 21-day fish assay for risk assessment. Endpoint sensitivity was analysised by compiling data from 142 individual studies for 21-day fish assay, 38 studies for fish sexual development test (FSDT) and 6 studies for fish full-life cycle test (FFLC), encompassing 62 chemicals with different MOAs. Conclusions from this analysis include: 1) vitellogenin (VTG), fecundity and gonad histology are the most sensitive endpoints for fathead minnow, medaka and zebrafish in 21-day fish assays; secondary sex characteristics (SSC) is a less sensitive endpoint and is likely inadequate to detect all known MOAs. 2) Results of biomarker endpoints like VTG and apical endpoints like fecundity from 21-day fish assay could be equally used for risk assessment. 3) Most of low observed effect concentrations (LOEC) were comparable for 21-day fish assay, FSDT and FFLC, which further support that data from 21-day fish assay can be used for risk assessement. However, significant difference in LOECs was also noted in some chemicals, suggesting that comprehensive endpoint sensitivity analysis is needed. This paper emphasizes that a weight of evidence approach is important to the decision making and testing strategy of EDCs.

PS 1538 MODELING THE IMPACT OF SHORT TERM LEAD EXPOSURE ON BLOOD LEAD LEVELS IN YOUNG CHILDREN.

J. Donohue[1], W. Mendez[2] and A. Shapiro[2]. *[1]Office of Water, U.S. EPA, Washington, DC and [2]ICF International, Fairfax, VA.* Sponsor: E. Doyle.

The Environmental Protection Agency (EPA) Integrated Exposure Uptake Biokinetic (IEUBK) Model can predict the risk of elevated blood lead levels in children exposed to environmental lead from a variety of sources. A major limitation of the model is its inability to predict the impact of short term or episodic increases in lead exposure on blood lead levels. The Office of Water (OW) contracted with ICF International to determine if the IEUBK model could be adapted so that it could predict changes in blood lead levels over timeframes consistent with a drinking water utility's quarterly monitoring program. ICF updated the background levels of lead in ambient air, soils, household dust, drinking water and diet used by the model. They devised an interface for the model that facilitated data entry and batch operation. The model was calibrated to ensure that the background blood lead projections (with typical drinking water exposure concentrations) were consistent with the distributions of blood lead levels seen in the Centers for Disease Control and Prevention's National Health and Nutrition Examination Survey (NHANES) monitoring program. The calibrated model has been used to estimate impacts on blood lead distributions in children exposed to increases in lead from drinking water. The model was found to provide consistent results for exposure averaging timeframes as short as three months. Monitoring under the EPA Lead and Copper Rule involves measuring lead concentrations in first-draw tap samples after the water has been stagnant for at least six hours which does not represent average human consumption. However, the adapted IEUBK model allows the OW to examine the incremental impact of hypothetical increases in average daily lead exposures on blood lead levels in young children and thereby inform projections of potential health risks. [The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

PS 1539 THE COUNCIL OF CANADIAN ACADEMIES EXPERT PANEL ON THE INTEGRATED TOXICITY TESTING OF PESTICIDES.

M. Trainer and L. Ritter[1, 2]. *[1]Council of Canadian Academies, Ottawa, ON, Canada and [2]School of Environmental Sciences, University of Guelph, Guelph, ON, Canada.*

Canada relies on well-established animal-based models and test systems to assess the safety and efficacy of chemicals and pharmaceuticals, including pesticides. Alternative testing strategies, including *in vitro* assays and "omics" technologies, represent an exciting opportunity for the development of tests that can evaluate a larger number of compounds, in systems that may more reliably predict potential adverse effects in humans. This approach also has the potential to identify the cellular mechanisms that may be the root cause of adverse effects. These new approaches may reduce the reliance on animal-based test systems and increase the efficiency and volume of testing, while maintaining the highest levels of scientific rigour. New legislation in the US and Europe will significantly increase the testing burden that cannot be met by the current toxicity testing paradigm. Integrated approaches for toxicity assessment may represent the means by which adequate safety testing, harmonized internationally, can address this challenge. The Pest Management Regulatory Agency (PMRA) of Health Canada requested that the Council of Canadian Academies convene an expert panel to evaluate the use of integrated testing strategies (IATA) for the regulatory risk assessment of pesticides. Pesticide formulations represent, on the one hand, one of the most data-rich chem-

ical groups in the field of regulatory toxicology while, on the other hand, one of the most data poor. To this end, they make an excellent model both for the development of new testing protocols and as a validation tool against which alternative testing strategies can be evaluated. Specifically, the 16-member panel is being asked to address the following questions: What is the current status of the use of integrated testing strategies by regulatory agencies around the world? What is the state of the science of integrated testing strategies? What are the potential impacts on the public's perception and confidence of IATA for pesticides?

PS 1540 THE PROS AND CONS OF USING DATABASES TO SUPPORT TOXICOLOGICAL DECISION MAKING.

T. Allio. *Office of Pediatric Therapeutics, U.S. FDA, Silver Spring, MD.* Sponsor: D. Jacobson-Kram.

This offering will provide a general overview of the use of databases in toxicological risk assessment and how understanding unique database structure will help safeguard against pitfalls in your project. Much of the information presented is derived from personal lessons learned over the years, with case examples in the area of genetic toxicology. An emphasis is placed on the need to incorporate user feedback into database development as well as an end user's ability to accomodate to new technologies as they develop. A review of several databases available to support decision making and translational research will be offered. Some tips on how to leverage the information derived from databases with data from empirical research and future areas for development will also be highlighted. Items presented in this offering are representative of the thoughts of the speaker and do not necessarily reflect the thoughts or policies of the FDA.

PS 1541 NEW RESULTS ON A UNIQUE PSYCHO-TOXICOGENOMIC DRUG SCREENING ASSAY TO ASSESS TREATMENT-EMERGENT SUICIDALITY RISK PRECLINICALLY.

D. Weissmann, L. Vincent, L. Cavarec, C. Plusquellec, C. Leborgne, F. Allemand and J. Pujol. *Biocortech, Paris, France.* Sponsor: E. Bush.

In recent years, there has been much focus on treatment-emergent suicidality - also referred to as drug-induced suicidality - with the FDA recently issuing draft guidance on how to assess this type of event in future trials. However, there is currently no way to predict this serious adverse event early in preclinical development. To possibly fulfill such a need, Biocortech set up an in vitro drug screening assay, exploiting a biological mechanism known as 'mRNA receptor editing' that has been shown, post mortem, to affect the post-transcription of serotonin 2c receptor mRNA in a specific way in suicide patients. The assay consists in measuring the changes of 5HT2cR RNA editing induced by screened drugs in chosen human cells and in comparing the drug-induced profiles with the ones associated to suicide and to interferon alpha treatments. On the basis of the results of a first screening of more than 65 molecules Biocortech entered into a collaboration with 7 pharmaceutical companies through a partnering with the Drug Safety Executive Council to perform, under blind conditions, the screening of 48 selected molecules belonging to several therapeutic classes. The screening was run at three concentrations, with molecules known to potentially induce suicidality and others for which such events had never been reported. The rounds of screening have shown that the assay was reproducible, showing some dose-effect relationships and that screened compounds could be clustered according to 4 patterns. The new results confirmed the potential utility of the 5HT2cR mRNA editing-derived drug screening to assess risk of treatment-emergent suicidality preclinically. According to those results induction of 5HT2cR editing profile mimicking those of suicide or of interferon alpha treatment by screened drugs could thus potentially constitute a risk factor of treatment emergent suicidality leading to the lowering of their priority for further development or to a closer clinical safety surveillance.

PS 1542 DERIVATION OF AN ORAL REFERENCE DOSE AND DRINKING WATER SCREENING LEVEL FOR SULFOLANE USING BENCHMARK DOSE MODELING.

C. Thompson[1], C. Perry[2], D. Gaylor[3], A. Tachovsky[2], B. Burkhalter[2] and L. Haws[2]. [1]*ToxStrategies, Inc., Katy, TX,* [2]*ToxStrategies, Inc., Austin, TX and* [3]*Gaylor and Associates, LLC, Eureka Springs, AR.*

Sulfolane is an organic solvent with a wide variety of industrial uses including sour gas sweetening, removal of hydrogen sulfide during shale and coal processing, the manufacture of polymers, electronics applications, and may also be found in pharmaceuticals as a residual contaminant remaining after the manufacturing process. Though sulfolane is not naturally occurring, it has been detected in groundwater near facilities where it has been used. Given the potential for human exposure (e.g., ingestion in drinking water, dermal contact during bathing and similar activities, and inhalation of sulfolane mist while showering) and the lack of any state or federal drinking water standards for sulfolane in the U.S., we employed benchmark dose (BMD) modeling to develop an oral reference dose (RfD) for sulfolane. Several studies involving oral exposures were identified and determined to be useful for development of toxicity benchmarks. Applying BMD modeling to several toxicological endpoints in these studies, RfD values ranging 0.012–0.037 mg/kg/day were calculated. The lower end of the range (0.01 mg/kg/day) was ultimately selected as the RfD in keeping with typical U.S. EPA practices. A drinking water screening level was subsequently developed based on this RfD using ingestion pathway equations and assumptions consistent with U.S. EPA's Regional Screening Levels (RSLs). This resulted in a drinking water screening level of 365 ppb. As a screening level, this value incorporates conservative assumptions to ensure an ample margin of safety and, as such, this value cannot be viewed as a bright line for adverse health effects, but rather provides the public with a reasonable degree of confidence that concentrations at or below 365 ppb are unlikely to impact their health. In contrast, concentrations above 365 ppb would need to be evaluated more closely to determine the likelihood for adverse health effects.

PS 1543 ASSESSMENT OF POPULATION HEALTH RISKS FROM ENVIRONMENTAL POLLUTION BY EMISSIONS OF A CHEMICAL WEAPONS DESTRUCTION FACILITY.

M. Kombarova[1], I. Lomteva[2], A. Radilov[1], E. Tsibul'skaya[1], N. Goncharov[1] and N. Khlebnikova[1]. [1]*RIHOPHE, Saint Petersburg, Russian Federation and* [2]*Institute for Applied Ecology and Hygiene, Saint Petersburg, Russian Federation.* Sponsor: R. Gupta.

The program of chemical weapons destruction, implemented in the Russian Federation as a signatory state of the Chemical Weapons Convention, envisions as one of its primary priorities safety provision of the population in regions potentially subject to an impact of Chemical Weapons Destruction Facilities (CWDFs). To assess the population health risks in protective activities zone of the CWDF in the Maradykovsky settlement (Kirov Region, Russian Federation), we developed and applied a computer program realizing the procedure, accepted in the Russian Federation and presented in the "Manual on Assessment of Human Health Risk from Environmental Chemicals" (R 2.1.10.1920-04, Moscow, 2004). The risk assessment procedure is based on two approaches: (1) direct risk assessment by the results of environmental monitoring and (2) risk assessment by emission dispersion modelling. Analysis of the design and engineering documentation and atmospheric emission inventory of the Maradykovsky CWDF allowed identification of priority air emission chemical pollutants: nitrogen, sulfur, and carbon oxides; alkali metal phosphates and sulfates; manganese compounds; C12-C19 alkanes, gasoline, fuel-oil ash; particulate matter; Russian VX (RVX), isobutyl methylphosphonic acid, 2-(diethylamino)ethyl isobutyl sulfide. The exposure scenario was found to be chronic inhalation, with the respiratory, sight, and digestive organs as the principal targets in the human body. Risk assessment on the basis of atmospheric air monitoring data revealed enhanced noncarcinogenic risks of exposure of respiratory organs to particulate matter (by a factor of 1.7) and to a complex of chemical pollutants, including nitrogen and sulfur dioxides and particulate matter (by a factor of 1.5).
Computer dispersion modeling of emissions of the Maradykovsky CWDF predicts acceptable chronic non-carcinogenic and carcinogenic risk levels.

PS 1544 INITIAL TOXICOLOGIC CHARACTERIZATION OF TRIAMINOGUANIDINIUM-1-METHYL-5-NITRIMINOTETRAZOLATE (TAG-MNT) IN FEMALE RATS AND *IN VITRO* ASSAYS.

L. R. Williams, C. J. Cao, E. LaFiandra, L. C. Crouse, M. A. Bazar, W. S. Eck and M. S. Johnson. *Directorate of Toxicology Health Effects Research Program (MCHB-TS-THE), U.S. Army Public Health Command (Provisional), Aberdeen Proving Ground, MD.*

Sustainable use of training ranges requires the development of compounds that have a minimal impact to the environment when used in a weapon system. Triaminoguanidinium-1-methyl-5-nitriminotetrazolate (TAG-MNT) is a military compound of interest for application in weapon systems. To ensure the health of potentially exposed personnel and the environment, several initial toxicity investigations were conducted and the results compared with another widely used energetic (hexahydro-1,3,5-trinitro-1,3,5-triazine; RDX). In a novel microplate Ames

assay, TAG-MNT was a weak mutagen and only mutagenic at the limit concentration of 2 g/L. However, TAG-MNT was cytotoxic to bacteria and a human liver cell line at 250 mg/L and greater. Unlike RDX, TAG-MNT did not have an affinity for the GABAa receptor convulsant site, and was predicted to not induce seizure. After oral acute dosing in female rats, TAG-MNT had no apparent adverse effect on the animals up to the limit dose of 2 g/kg. However, daily oral dosing for 14 days at exposures of 1000 mg/kg-d and above caused reduction in food intake, weight loss, increased kidney weight, leucopenia, and elevated blood urea nitrogen and creatinine levels. Leucopenia, increased liver mass, evidence of liver hepatocyte necrosis and centrilobular hypertrophy were observed at 500 mg/kg-d and above. TAG-MNT was negative in the rat micronucleus assay of blood samples. Based upon these data, the 14-day oral NOAEL is 250 mg/kg-d.

PS 1545 HUMAN HEALTH RISKS FROM EXPOSURE TO 1, 4-BUTANEDIOL IN CRAFT KIT BEADS.

R. Mattuck, M. Seeley, K. R. Reid and J. E. Goodman. *Gradient, Cambridge, MA.*

Aqua Dots is a craft kit that allows children to create multi-dimensional designs using small colored beads that fuse together when sprayed with water. Due to a manufacturing error, the beads in certain kits contained 1,4 butanediol (1,4-BD) instead of 1,5-pentanediol. In humans, 1,4-BD is metabolized to γ-hydroxybutyric acid (GHB), high doses of which can lead to a sedative effect. We evaluated potential exposures to 1,4-BD in Aqua Dots beads following reports that two children who had ingested large quantities of the beads experienced a temporary loss of consciousness. Laboratory tests were conducted to quantify the 1,4-BD removed from the beads under different exposure scenarios. We used a wipe test to evaluate dermal contact; dissolution tests in simulated saliva, gastric, and intestinal fluids to evaluate ingestion; and a volatilization test to evaluate inhalation. The beads contained an average of 12 mg 1,4 BD per bead. 1,4-BD was not detected in the volatilization test from either wet or dry beads at room temperature. We estimated intakes of 1,4-BD from direct ingestion of beads; mouthing of beads; and dermal contact with dry or wet beads, followed by dermal absorption or incidental ingestion of 1,4-BD from hand-to-mouth activity. We assumed a child would play with the kit every day for two weeks and estimated 1,4 BD intakes under typical, moderate, and high exposure scenarios. Intakes were compared to critical effect levels for acute CNS effects (LOAEL of 40 mg/kg for loss of consciousness in humans) and subacute/ subchronic systemic effects (NOAEL of 50 mg/kg-day for mild liver effects in rats). "Typical" (0.67 mg/kg-day) and "moderate" (2.9 mg/kg day) intakes were well below critical effect levels. The "high" intake, a single ingestion of 36 beads for a 2-year old (37 mg/kg), was comparable to the LOAEL for acute CNS effects.

PS 1546 SAFETY OF COMPOUNDS RELEASED FROM DEGRADING CARPET MATERIALS IN SCHOOL BUILDINGS.

B. J. Kelman[1], M. W. Krause[1], L. J. Swenson[2] and C. A. Robbins[1]. [1]*Veritox, Inc., Redmond, WA and* [2]*Veritox, Inc., Portland, OR.*

Complaints of upper respiratory irritation from staff and students in a new school building led to evacuation of the building for more than four months. An investigation which included extensive air sampling identified methyl and ethyl hexanols which had either not been reported or rarely reported in health-oriented scientific literature. We identified a few reports of ethyl hexanols being released from carpeting in contact with high moisture, alkaline cement floors from existing materials science literature. We also identified very limited reports of ethyl hexanols being associated with irritating atmospheres from existing toxicology literature. We were unable to identify any data on 4 and 5 methyl hexanols from in vitro or animal testing. We hypothesized that ethyl hexanols were the source of irritation. To test this hypothesis, we collected air samples and analyzed them using a TO-17 protocol modified to include hexanols. Our samples contained little ethyl hexanol, but 4- and 5-methyl hexanols at concentrations as high as 17 ppb as well as low levels of other volatile organic compounds (VOCs). Methyl hexanols were present at highest concentrations in rooms containing carpeting and at low to non-detectable levels in rooms without carpeting. Complaints coincided with the presence of carpeting. A sample of reagent grade 5-methyl hexanol produced the same subjective complaints as experienced in the school building. With very few exceptions total VOCs were less than levels generally associated with degraded indoor air quality. Carpeting, mastic, and sealant were removed from building floors and replaced with products designed to withstand the degrading effects of moist, alkaline cement. No hexanols were detected in indoor air samples following remediation and the school was successfully reoccupied. Our data indicate that 4- and 5-methyl hexanols are irritating to humans at very low levels and that the inhalation toxicology of these compounds should be investigated. Work supported by Seattle Public Schools.

PS 1547 HEPATOTUMORIGENICITY OF ETHYL TERTIARY-BUTYL ETHER (ETBE) BY INHALATION EXPOSURE BUT NOT ORAL ADMINISTRATION IN F344 RATS.

K. Nagano[1], T. Nishizawa[1], K. Yamazaki[1], T. Noguchi[1], A. Hagiwara[2], F. Nishimaki[3], S. Iida[3], K. Komiya[3] and S. Fukushima[1]. [1]*Japan Bioassay Research Center, Japan Industrial Safety and Health Association, Kanagawa, Japan,* [2]*DIMS Institute of Medical Science, Nagoya, Japan and* [3]*Japan Petroleum Energy Center, Tokyo, Japan.*

To achieve the Kyoto Protocol, the Petroleum Association of Japan decided to use ETBE as a gasoline blending component from 2010. Therefore, carcinogenicity of ETBE was evaluated prior to nationwide use. 1) Carcinogenicity of ETBE was examined by inhalation exposure and oral administration using F344/DuCrlCrlj rats. In the inhalation study, groups of 50 male and 50 female rats were exposed to ETBE at 0, 500, 1500 or 5000 ppm (v/v) in whole-body inhalation chambers for 6 hrs/day, 5 days/week for 104 weeks. Significant increase in the incidence of hepatic tumors was observed in males exposed to 5000 ppm (control: no tumors, 500 ppm: 2 rats with hepatocellular adenoma, 1500 ppm: 1 rat with hepatocellular adenoma, 5000 ppm: 9 rats with hepatocellular adenoma and 1 rat with hepatocellular carcinoma). On the other hand, no significant increase in the tumor incidence was found in females at any dose of ETBE. In the oral study, groups of 50 male and 50 female rats were given drinking water containing ETBE at doses of 0, 625, 2500 or 10000 ppm (w/w) for 104 weeks. No significant increase in the incidence of tumors was detected in any organ of either males or females. Present studies indicate that ETBE exerts tumorigenicity in the liver of male rats by inhalation exposure, but no effect by inhalation in females and oral administration in both male and female rats. 2) ETBE was negative in the rat micronucleus test performed with bone marrow, and showed promotion activity in the liver using rat medium-term multi-organ carcinogenesis bioassay. In conclusion, ETBE is nongenotoxic due to the micronucleus test and with reference to the literature examining genotoxicity, and exhibits weak carcinogenicity in the liver after the inhalation exposure but not oral administration in rats.

PS 1548 GENOTOXITY OF FURAN IN F344 RATS USING THE *IN VIVO* COMET AND MICRONUCLEUS ASSAYS.

W. Ding[1], R. H. Heflich[1], M. G. Pearce[1], G. White[2], R. A. Mittelstaedt[1], J. G. Shaddock[1], P. L. McDaniel[1], D. R. Doerge[3], M. G. Manjanatha[1] and A. Aidoo[1]. [1]*DGMT, U.S. FDA/NCTR, Jefferson, AR,* [2]*Toxicology Pathology Associates, U.S. FDA/NCTR, Jefferson, AR and* [3]*DBT, U.S. FDA/NCTR, Jefferson, AR.*

IARC considers furan as reasonably anticipated to be a human carcinogen. To understand its cancer mode of action, we evaluated the genotoxicity of furan in rats using the in vivo Comet and micronucleus (MN) assays. Based on furan's metabolism, we hypothesized that induction of oxidative DNA damage may be partially responsible for its genotoxicity. Thus, in addition to the standard alkaline Comet assay, we performed the Fpg- and Endo III-modified versions of the assay. Also, since in vivo Comet assay results are affected by the sampling time, we conducted time-course experiments in which F344 male rats were treated by gavage with 16 mg/kg furan (twice the daily dose given in cancer bioassays) at 0, 24, 48 and 72 hr. Animals were sacrificed 1, 3, 6, 8, 16 and 24 hr after the last dose. Liver (cancer target tissue) and bone marrow (nontarget tissue) were collected for the Comet assay; peripheral blood was collected for the MN assay. Positive responses were observed with the Comet assay in liver, but not in bone marrow. The maximum response for the standard and Fpg-modified Comet assay was at 1 hr, while the maximum for the Endo III-modified assay was at 3 hr. The MN responses were negative for all time points. A concentration-dependent study will be conducted to address the question of whether the Comet assay can be used to measure DNA damage induced by cancer bioassay doses of furan. Oxidative DNA damage is closely related to inflammation, which is associated with carcinogenesis, especially in the promotion phase. Thus, our observations of the elevated direct and oxidative DNA damage in furan-treated rat liver suggest mechanisms by which furan may act as a carcinogen in rat liver. The data also indicate that the genotoxicity of furan in the rat is targeted to the liver and that the liver Comet assay provides a useful complement to the peripheral blood MN assay in detecting genotoxic responses.

PS 1549 ACUTE ORAL TOXICITY STUDIES OF A NEW PESTICIDE-AMTS18 IN MICE.

J. Zhang, Y. Pang and S. Brimijoin. *Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN.*

A recent paper from our laboratories (Pang Y-P, PLoS ONE 4(2): e4349, 2009) reported a newly developed methanethiosulfonate-compound, AMTS18 that could selectively and irreversibly inhibit insect acetylcholinesterase (AChE). Studies in

vitro with this potentially insect-selective pesticide showed that exposure to 6 micromolar concentrations for 1 hr caused 99% inhibition of the AChE in greenbug aphids (shizaphus graminum) but less than 2% inhibition of the human RBC AChE. Although AMTS18 is not yet potent enough for field use we decided to determine whether methanethio-sulfonates in general should be expected to exhibit unacceptable non-specific toxicity in mammals. For the purpose of this acute toxicity study, male ICR CD1 mice were gavaged with a dose of 33mg/kg, 100mg/kg or 300mg/kg AMTS18 in corn oil and, 3 hr later, blood samples were collected to test plasma AChE activity. At the same point a functional observational battery test (FOB) was carried out to evaluate neurological and behavioral effects. Exactly 24hr later, the FOB test was repeated, and terminal blood samples were collected for assays designed to detect toxicity in liver (alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transpeptidase, kidney (plasma urea nitrogen or BUN and creatinine), and heart (creatine kinase-MB). Plasma levels of glutathione (known to react with methanethiosulfonates) and AChE activities were also assayed. The results of this study showed no mortality any of AMTS18 groups, hence LD50 could not be determined, and none of the sentinel markers exhibited statistically significant changes (as evaluated by one way analysis of variance). Our FOB studies likewise failed to show any significant effects. On the basis of these findings AMTS18 exerts no detectable toxicity even at the high dose of 300mg/kg delivered by the gavage route. We conclude that it is worth exploring equally selective but more potent methanethiosulfonates as insect-targeting pesticides with reduced risk to mammals.

PS 1550 SKIN BIOCOMPATIBILITY TESTING OF A POLYISOPRENE ELASTOMER.

N. Pechacek, T. Brunshidle and L. Eichinger. *3M, St. Paul, MN.*

Elastomer materials (elastomers) have numerous commercial applications due to their extensibility, stretchability, and durability. Sources of these materials can be natural, synthetic, or a hybrid of natural and synthetic. Elastomers frequently have applications that involve significant skin contact. Historical use experience by industry has shown that various elastomers can pose notable skin hazards (e.g., allergic contact dermatitis, primary and/or cumulative skin irritation). Subsequently, uses of elastomers in products that result in significant skin contact necessitate a thorough assessment of their skin biocompatibility prior to commercialization. To conduct such an assessment, ISO Standard 10993, Biological Evaluation of Medical Devices, Part I for surface devices with skin contact; served as guidance for evaluating a polyisoprene elastomer proposed for use in a 3M product with significant skin contact. The skin biocompatibility assessment consisted of three assays: an in-vitro cytotoxicity: L929 agar overlay test (cytotoxicity assay); an acute dermal irritation test in rabbits (dermal irritation assay); and a local lymph node assay in mice (LLNA). The cytotoxicity assay provided an assessment of the potential cytotoxicity of leachable materials from the elastomer. The dermal irritation assay was an in vivo primary dermal irritation assay modified to incorporate repeat skin contact for 7 hours/day for 5 consecutive days to assess both primary and cumulative irritation of the neat elastomer. Lastly, the LLNA was conducted to provide an evaluation of the dermal sensitization potential of extractables from the elastomer. Under the study conditions, no appreciable cytotoxicity, irritation, or sensitization was observed. Subsequently, it is concluded that the polyisoprene elastomer is unlikely to have appreciable skin hazards.

PS 1551 CONSIDERATIONS FOR DEMONSTRATING THE INTER-LABORATORY RELIABILITY OF CHORIOALLANTOIC MEMBRANE VASCULAR ASSAY (CAMVA) AND THE BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY (BCOP).

G. Mun[2], N. Wilt[2], D. A. Donahue[1], J. Avalos[1], K. Norman[2], A. Hilberer[2], F. A. Simion[1] and H. Raabe[2]. *[1]Research and Development, Kao Brands Company, Cincinnati, OH and [2]Institute for in vitro Sciences, Inc., Gaithersburg, MD.*

In vitro assays evaluating ocular irritation potential are routinely used by personal care companies. Two of these in vitro assays are the Chorioallantoic Membrane Vascular Assay (CAMVA) and the Bovine Corneal Opacity and Permeability Assay (BCOP). These assays do not require the use of live animals, provide reliable predictive data and are rapid to conduct. The BCOP uses excised bovine corneas to predict ocular irritation. The CAMVA uses vascular network of fertilized chicken eggs as a conjunctival model to predict ocular irritation. Both BCOP and CAMVA have been used for over fifteen years for product development, workplace safety, and safety claims substantiation. This poster describes procedures and considerations for demonstrating the inter-laboratory reliability of the BCOP and CAMVA. For Kao Brands Company, a large BCOP and CAMVA historical database exists that covers multiple consumer product categories such as hair shampoos, skin cleansers, and hair styling sprays (containing ethanol). Therefore, a proper audit of candidate contract laboratories is important for seamlessly generating consistent results. First, candidate contract laboratories were audited for technical capabilities and GLP compliance. Next, reference materials with known BCOP and CAMVA data were tested at each new laboratory for verification of proper assay performance. After qualifying a contract laboratory, assay data generated from additional samples have been examined (results should be within the historical ranges of similar products) for additional assurance. The consistency of new data indicates that a detailed qualification process of new contract laboratories is important in generating accurate data and maintaining a high quality database.

PS 1552 SAFETY EVALUATION OF β-HYDROXY CYCLODEXTRIN IN WISTAR RATS.

R. Nirogi, V. Goyal, R. Vedamurthy, M. A. Mulla, S. Pandey, S. Jana and A. Gothi. *Toxicology, Suven Life Sciences Limited, Hyderabad, Andhra Pradesh, India.* Sponsor: V. Reddy.

n preclinical safety assessment of potential new drugs, it is required that the material of interest must be suitably formulated in a manner that allow adequate administration of the test substance with little or no effects in test animals that is attributable to the vehicles used in preparing such formulations. There are many vehicles that have been used in preclinical formulation. B-Hydroxy Cyclodextrin (BCD) is one of the most commonly used vehicles to solubilize and stabilize the test substance for formulation preparation in preclinical studies. The present study was planned to evaluate the oral toxicity of BCD in Wistar rats. Three groups of Wistar rats (4/sex/group) were dosed orally at 500, 2000 and 4000 mg/kg/day of BCD and one control group was dosed with distilled water for 14 consecutive days. No treatment-related clinical signs were noticed up to 4000 mg/kg although loose feces were observed during fasting before terminal sacrifice on day 15 at 2000 and 4000 mg/kg/day. Body weight and feed consumption were slightly decreased in male rats at 2000 and 4000 mg/kg/day but these changes did not reach statistical significance. No changes were observed in any hematological, biochemical or urine parameters after 14 days of treatment except minimal increase in ALP level in female rats at 2000 and 4000 mg/kg. No abnormalities were found at necropsy that could be attributed to the treatment. On microscopic examination, no treatment-related alterations were observed in examined organs and tissues. In conclusion, transient diarrhea and slight reduction in body weight and feed consumption were the only treatment-related effects. These changes are well-known physiological responses to the presence of high amounts of non-digested, fermentable carbohydrates in the lower gut. Based on these results, it is inferred that BCD can be administered up to 4000 mg/kg/day without any apparent effect on health of animals.

PS 1553 ICCVAM RECOMMENDATIONS ON THE USEFULNESS AND LIMITATIONS OF THE CYTOSENSOR MICROPHYSIOMETER® (CM) TEST METHOD FOR OCULAR SAFETY TESTING.

J. Merrill[1], D. Lowther[1], A. Layton[2], J. Redden[3], M. Wind[4] and W. Stokes[5]. *[1]U.S. FDA, Silver Spring, MD, [2]U.S. CPSC, Bethesda, MD, [3]U.S. EPA, Washington, DC, [4]U.S. CPSC (Retired), Bethesda, MD and [5]NICEATM, NIEHS, Research Triangle Park, NC .*

ICCVAM recently evaluated several in vitro test methods as potential replacements for the rabbit eye test for identifying potential ocular hazards. None of the methods were considered adequate as complete replacements. However, ICCVAM concluded that test substances within a defined limited applicability domain (water soluble surfactants, surfactant-containing formulations and nonsurfactants) that are positive for severe effects in the CM can be classified as ocular corrosives/severe irritants (EPA Category I, EU R41, GHS Category 1). False positive rates ranged from 0% (0/17, 0/18) to 10% (3/29) and false negative rates from 9% (2/23) to 50% (6/12) depending on the hazard classification system used. ICCVAM also concluded that test substances within an even more restricted applicability domain (water soluble surfactant chemicals and certain types of surfactant-containing formulations) can be considered as not classified for ocular hazards (EPA Category IV, EU Not Labeled, FHSA Not Labeled) without any further testing if they are negative in the CM. Although false positive rates were high (50% [3/6] to 69% [18/26]), false negative rates ranged from 0% (0/27, 0/28, or 0/40) to 2% (1/42 or 1/47) depending on the hazard classification system used. A chemical that produces a response in CM between these two extremes would require additional testing (in vitro and/or in vivo) to establish a definitive classification. The CM test method is not considered adequately valid for identification of mild or moderate ocular irritants (GHS Categories 2A/2B; EU R36; EPA Categories II/III). ICCVAM also recommended a standardized CM protocol and future studies to expand the applicability domain of the CM. These recommendations have been forwarded to Federal agencies and if accepted, the CM will be the first in vitro test method available in the U.S. for identifying substances that do not require ocular hazard labeling.

PS 1554 L'ORéAL COMMITMENT IN THE DEVELOPMENT, EVALUATION, AND VALIDATION OF SCREENING AND TESTING APPROACHES CONTRIBUTING TO THE 3RS.

J. R. Meunier, N. Alépée, J. Cotovio, D. Duché, G. Ouedraogo, L. Marrot, S. Teissier, J. M. Ovigne and J. Leclaire. *L'Oréal, Aulnay sous Bois, France.* Sponsor: H. Toutain.

Dissemination of advances on alternative methods represents a step to promote alternatives to animal testing in line with the EU Cosmetics Directive. Over the past decade there have been great efforts in the industry or academic laboratories to develop alternative methods either in silico or in vitro that would comply with regulatory constraints. The implementation is still limited given that it is a great challenge to replace historical approaches by good and well predictive in vitro tests. L'Oréal has, based on these principles, developed test methods to screen and test potential effects on chemicals. We have focused initially on approaches for skin irritation, since this is an important endpoint for chemicals used in Cosmetics. A peer review on various aspects of alternative techniques was performed at all stages of the research and development with a focus on in vitro methods improvement of chemicals selection (screening) as well as quality testing. Following the development of the screening test, we have implemented some protocols (i.e. EpiSkin and SkinEthic RHE) for pre-validation and validation processes according to ECVAM's recommendations. To ensure quality and objectivity, experts from international committees oversee the content. Details of the approach will be presented for both skin corrosion and irritation with a set of 20 reference chemicals. Moreover, the poster described the practical approaches developed by L'Oréal in the areas of eye irritation (SkinEthic HCE defined with 90 chemicals), skin sensitization (MUSST assay optimized with 50 chemicals), phototoxicity and genotoxicity. By participating actively in relevant forums, L'Oréal and other industries continue to promote the development of new tools and methodologies as well as favor the acceptance of in vitro alternatives by authorities.

PS 1555 TOXICITY OF SMALL 50CC ENGINE EMISSIONS ON ORGANOTYPIC CULTURES OF RAT LUNG TISSUE: 2-STROKE, 4-STROKE ENGINES AND LUBE OIL (LO) QUALITY IMPACTS.

J. Morin[1], D. Preterre[2], V. Keravec[2], C. Monteil[1] and F. Dionnet[2]. [1]*U644, INSERM - School of Medicine Pharmacy, Rouen, France and* [2]*Certam, Saint Etienne du Rouvray, France.* Sponsor: R. Forster.

Emissions small scooters is becoming a major health concern in Asia, Africa and in European cities. We aim to compare the impacts on the toxicity profiles according to engine technology, exhaust after-treatment and LO quality.
One 4-stroke and two 2-stroke scooters (50 cc bore) were run on dynamic benches EC47 cycle. Emissions were continuously monitored, sampled and hot-diluted prior being delivered to organotypic cultures of lung tissue under Air/liquid conditions for 3 hours. Lung tissue was evaluated for viability (ATP), anti-oxydant defenses (GSH, Catalase, SOD, GST, GPx, and GRED activity levels) and inflammation (TNFalpha secretion). 4-stroke engine had a moderate impact on lung tissue ATP, induced a marked GSH depletion and increased GPx activity. 2-strokes engine impacts varied according to after-treatment technology and LO. Low oxidation catalysis and mineral oil were found to be the worse situation inducing a marked ATP and GSH depletions, decreased activity of both Mn and Cu/ZnSOD and a decrease in TNF alpha secretion. High oxidation catalysis and semi-synthetic LO was found to be the best situation with only minor impacts on the above listed endpoints. Measurements of CO, NO, NO2, HC, and particulate matter (PM) showed that under warm engine conditions, 2-stroke engine with high oxidation catalysis and semi-synthetic LO was the least emitting situation for CO, HC and PM. 4-stroke engine proved to have intermediate emissions levels for CO, HC and PM. 2-stroke engine with low oxidation catalysis and mineral LO was found to be the worse situation for HC and PM. In conclusion : Emissions from small scooters may have detrimental impacts on lung tissue which may be highly reduced by the use of an oxidation catalyst and high quality LO. Suitable recently designed after-treatment strategies allow a very efficient reduction of 2-stroke engine emissions and toxic potential leading to an even lesser impact than for the 4stroke commercially available engine emissions.

PS 1556 HISTORY OF SAFE USE OF CRY1C PROTEIN.

R. Ranjan[1] and C. Herouet-Guicheney[2]. [1]*Regulatory Toxicology, BayerCrop Science LP, Durham, NC and* [2]*Regulatory Toxicology, Bayer SAS, Bayer CropScience, Sophia Antipolis, France.*

Plants expressing insecticidal Bt proteins (δ-endotoxin) are called Bt protected plants and are important in modern agriculture. These plants provide protection from insect pests and reduce the use of chemical pesticides. Due to high specificity of their mode of action, Cry proteins are not toxic to non-target organisms including insects, animals and human beings. We have examined the history of safe use of Cry1C protein and assessed its safety concern based on review of available literature which contains data from biosafety studies. In-silico homology search did not show similarity of Cry1C protein to known allergen or toxins. Sequence search did reveal 50% homology with other Cry1 proteins, especially to Cry1Ab, Cry1Ac and Cry1F which have been used in many commercial biopesticides and Bt crops. In-vitro study using simulated gastric fluid demonstrated that Cry1C protein was readily degraded at pH 1.2, and in heat stability study completely degraded at 100°C. In addition, animal feeding studies and allergenicity studies with plant products expressing Cry1C protein did not show treatment related effect. In conclusion, there is a reasonable certainty of no harm in inclusion of Cry1C protein in human food or animal feed.

PS 1557 LOCAL AND SYSTEMIC TOXICITY OF IMPLANTED ACCELERATOR-FREE POLYCHLOROPRENE-TYPE AND LATEX SURGICAL GLOVE MATERIAL.

M. Goravanahally, A. F. Hubbs, P. H. Nicolaysen, M. L. Kashon, L. A. Battelli, B. F. Law, P. A. Willard and P. D. Siegel. *HELD, National Institute for Occupational Safety and Health, Morgantown, WV.*

Asthma from latex gloves has prompted marketing of non-latex gloves including synthetic polyisoprene and polychloroprene surgical gloves. We have recently reported the presence of dihydroabietic (DHA) acid in these gloves, a compound that is extremely toxic in fish, but uninvestigated in mammals. Potential toxicity of DHA in surgical gloves is a concern because of potential exposure of patient tissues to DHA during extensive surgical procedures. Therefore, we investigated the hypothesis that the subcutaneous implantation of DHA containing polychloroprene gloves causes local and systemic toxicity. Mice (6/group) were subcutaneously implanted with 200 mg each of a polychloroprene glove or of a latex glove or 9 mg DHA, or underwent sham surgery, and were sacrificed 24 hr post-exposure. Implants were recovered for DHA analysis and tissues were processed for semi-quantitative histopathology. Latex caused multifocal and coalescent, moderate to marked necrosis of myofibers at the implantation site in all mice (pathology score, 7.16 ± 0.16; p=0.0048) and mild to moderate suppurative inflammation (5.66 ± 0.42; p=0.0048). The polychloroprene caused multifocal, mild to moderate, necrosis and mild inflammation in 3 of 6 mice. In the tissues beneath the implantation site (the epaxial myofibers and the adjacent fascia), significant changes were observed that varied by tissue and glove material. In peripheral blood, latex increased polymorphonuclear cells (37% ± 0.01; p<0.0001) and decreased lymphocytes (61% ± 0.01; p<0.0001) compared to sham controls. In bone marrow cytology, polychloroprene material decreased the myeloid to erythroid ratio (p=0.0767) and decreased the mature pool of myeloid cells in bone marrow sections. These results suggest the need for additional studies of local cytotoxicity and systemic hematotoxicity of surgical glove components.

PS 1558 DETAILED CHEMICAL COMPARISONS OF NEW AND OLD SERIES REFERENCE SMOKELESS TOBACCO PRODUCTS (STP).

J. H. Lauterbach[1] and D. A. Grimm[2]. [1]*Chemistry & Toxicology, Lauterbach & Associates, LLC, Macon, GA and* [2]*Coordinated Instrumentation Facility, Tulane University, New Orleans, LA.*

Some health experts recommend that smokers, who refuse to quit or refuse to use nicotine replacement therapies, switch to low TSNA smokeless tobacco products (STP). US-style moist snuff is the most popular STP, but has attracted criticism because of toxicological concerns. Use of *in vitro* assays (e.g., Ames; Rickert et al., Regul. Toxicol. Pharmacol. 2009 53:121-33)) to assess STP toxicity was of limited utility in distinguishing product types and brands within a type; and use of the *in vitro* mammalian GreenScreen HC assay (GADD45a-GFP reporter hosted in TK6 cells) (Lauterbach et al., Toxicol. Sci. 2010 114(1-S):2590) did not yield data that correlated with levels of known genotoxicants in the old series reference STP. However, the genotoxicity may stem from other sources such as sugar-amine reaction products as indicated by detailed GC-MS analyses of the STP. A new series of reference STP has just been released, and the moist snuff (CRP2 replaces 2S3), dry snuff (CRP3 replaces 1S2),and chewing tobacco (CRP4 replaces 2S1) products use the same recipes as did the earlier samples. While the routine analytical data on the new products are similar to those of the old, the detailed analytical data (GC-MS analyses (BSTFA/DMF extract of STP) were performed on an Agilent 6890 GC coupled with an Agilent 5972 MS with DB-5MS capillary GC column (25 m X 0.50 ?m film thickness and 0.25 mm ID)) was used. Our research shows differences in sugars, organic acids, and marker compounds for sugar-amine reaction products. Our findings indicate that toxicological properties of the new reference STP and

their performance on toxicological assays may not be the same as those of the older reference STP with likely higher genotoxicity (based on levels of marker compounds for sugar-amine reaction products) found in the CRP2 and CRP3 samples than in their older counterparts.

PS 1559 IN-DEP EVALUATION OF THE VARIABILITY IN CHEMICAL COMPOSITION OF CHAMOMILE ESSENTIAL OIL (CEO) DUE TO GEOGRAPHICAL ORIGIN OF THE SOURCE PLANT: IMPLICATIONS FOR SAFETY EVALUATION.

R. Persaud[1], T. Re[1], V. Srinivasan[1], E. Antignac[2] and G. Nohynek[2]. *[1]Product Safety, L'Oreal USA, Clark, NJ and [2]Global Safety Evaluation, L'Oreal R&D, Asnieres, France.*

The major determinant of the chemical composition in any plant is its genotype. In addition, other factors such as geographic origin, age, crop management practices, and post-harvest manipulation can eventually affect the composition of extracted material such as essential oils. The geographic origin is one of the key factors capable of significantly altering the chemical composition of the CEO (Matricaria recutita L., commonly known as German chamomile). It has at least 12 different chemotypes originating from countries in Europe, Asia and South America that vary considerably in terpenoid and flavonoid concentrations with only minute differences in coumarin and phenolic acid content. An extensive review of the literature was done on CEO to assimilate information on chemical composition for the 12 plant origins, focusing on the levels of flavonoid and terpenoid actives. Due to the lack of toxicity data on most of the major components of CEO it was necessary to implement an in silico approach. The toxicity class was estimated by the Cramer rules using ToxTree. Structural alerts were evaluated with DEREK and read-across toxicity information on structurally-related compounds was performed utilizing OECD Toolbox. The octanol-water partition coefficient of the compounds was assessed with EPISUITE and used with molecular weight to estimate dermal penetration. The chemical with the major safety concern was most critical in determining the acceptable use level of CEO. The results of our in silico evaluation demonstrates that the CEO from Greece has the lowest level of highly toxic compounds (Cramer Class III) thus the highest concentration of acceptable use. However, that from Moldova has the highest amount of highly toxic constituents and should therefore be permitted at lower levels in cosmetic or personal care products. This implies an essential oil should have an independent safety assessment for each geographic origin of its plant source.

PS 1560 ORAL (GAVAGE) ONE-GENERATION REPRODUCTION STUDY OF CITRONELLYL NITRILE IN RATS, WITH AN EVALUATION THROUGH SEXUAL MATURITY IN THE F1 GENERATION.

J. Scognamiglio, D. McGinty, V. T. Politano and A. Api. *Research Institute for Fragrance Materials, Woodcliff Lake, NJ.*

Male and female rats (25 rats/sex/dose) were administered citronellyl nitrile (CAS# 51566-62-2) at dosages of 0, 75, 200, and 500 mg/kg/day. Administration via gavage of the test material or the vehicle, corn oil, began before the cohabitation period (83 days for males; 14 days for females), through cohabitation (7-13 days), until the day before sacrifice (males) or day 25 of presumed gestation (females that do not deliver) or day 4 postpartum (females that deliver a litter). F1 generation rats selected for continued evaluation were sacrificed on day 60 (± 3) postpartum. There were no treatment-related deaths at any dosage level tested. P generation male rats at 500 mg/kg/d and P generation females at 200 and 500 mg/kg/d experienced slight to extreme excess salivation was observed over the treatment period. Mean body weights, body weight gains and absolute and relative feed consumption values were comparable among the dosage groups. There were 24, 22, 25, and 23 pregnant rats in the four dosage groups; all pregnant rats delivered litters. The number of pups found dead or presumed cannibalized during lactation days 1-5 was slightly increased in the 500 mg/kg/day dosage group; consequently, the viability index (number of live pups on lactation day 4/ day 1) was reduced. All other natural delivery and litter observations were comparable. There were no treatment-related clinical signs, gross lesions or changes in body weight, body weight gains, feed consumption or organ weights in the male and female F1 generation rats at any dosage level tested. Based on the results of this study, the no-observable-adverse-effect-level (NOAEL) for toxicity of citronellyl nitrile is 500 mg/kg/day. The reproductive NOAEL in the P generation rats and the NOAEL for viability and growth of the F1 generation offspring is greater than 500 mg/kg/day.

PS 1561 REPEAT ORAL DOSE TOXICITY OF MELAMINE IN RATS.

X. Mu, Q. Kong, H. Yu, J. Zhou, H. Xu, X. Yang and Y. Jiang. *WuXiAppTec (Suzhou), Suzhou, China.*

Melamine is an important and widely used organic industrial chemical. Recently, clinical findings of renal failure and kidney stones in infants have been associated with the ingestion of melamine contaminated infant formula. To understand the toxicity and clinical outcome of melamine exposure, two repeat oral dose studies in rats were performed to characterize the sub-chronic toxicity of melamine. The first rat study was a 14 day oral study followed by an 8-day recovery period. The dose levels were 140, 700, and 1400 mg/kg/day (lowered to 1000 mg/kg/day subsequently due to mortality). Assessment of toxicity was based on mortality, clinical signs, body weights, ophthalmic findings, clinical pathology, gross pathology, organ weights, and microscopic observations. Oral administration of melamine at 700 mg/kg/day for 14 consecutive days in rats produced compound related clinical signs (red urine), decreased body weights, and changes in clinical pathology (increased BUN and CRE) and anatomical pathology (renal tubular cell debris and crystals deposition and hyperactive regeneration of renal tubular epithelium). The kidney was identified as the target organ. Oral administration at 1400 mg/kg/day (subsequently lowered to 1000 mg/kg/day) resulted in animal death and moribundity. There were no treatment related findings in the 140 mg/kg/day group. There were no compound related findings in the high dose recovery animals. In the second rat study, genomic biomarkers were assayed in the kidney tissues after 5 day repeat oral dose with melamine. At 1050 mg/kg/day, similar clinical and anatomical pathology findings as described above were observed. The genes measured, Kim-1, Clu, Spp1, A2m, Lcn2, Tcfrsf12a, Gpnmb, and CD44, were significantly up-regulated (5 to 550 folds), while Tff3 was significantly down regulated (5 folds). The gene expression of Cystatin C (a urinary biomarker for glomerular damage) was hardly changed. These results indicated that genomic markers could sensitively diagnose melamine induced kidney injury.

PS 1562 REPRODUCIBILITY OF METABOLOMICS PERFORMED IN PLASMA SAMPLES OF 28 DAY RAT SYSTEMIC TOXICITY STUDIES.

H. G. Kamp[1], E. Fabian[1], M. Herold[3], M. Kapp[1], G. Krennrich[2], R. Looser[3], W. Mellert[1], A. Prokoudine[3], V. Strauss[1], T. Walk[3], J. Wiemer[3] and B. van Ravenzwaay[1]. *[1]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany, [2]Computational Chemistry, BASF SE, Ludwigshafen, Germany and [3]Metanomics GmbH, Berlin, Germany.* Sponsor: C. Shelp.

Metabolomics is a tool to measure treatment-induced changes of endogenous metabolite levels in biological samples. BASF and metanomics have developed a large metabolomics data base (MetaMap®Tox) containing the metabolome of approximately 500 data rich chemicals, agrochemicals and drugs. This metabolome-database has been built based upon 28 day studies in rats (adapted to OECD 407 guideline) with blood sampling and metabolic profiling after 7, 14 and 28 days after test substance treatment. Besides the reference data base, reproducibility is of major importance to allow for a reliable application of metabolomics in toxicological in vivo testing. We have previously shown the robustness of the metabolomics technique applied by BASF, displayed by constancy of variability in control groups over time. In order to assess the intra-laboratory reproducibility under treatment with test substances, we have tested > 10 reference compounds at least twice. For these compounds, the animal experiments as well as the metabolome analysis have been performed independently under the same conditions. Statistical correlation analyses showed that the repeated treatment induced similar changes to the metabolome. Moreover, when compared against the MetaMap®Tox data base, the repetitions in almost every case occurred in direct sequence in a correlation-based ranking. Based on these results, it can be concluded, that metabolomics can reproducibly be applied during toxicological in vivo testing in rats.

PS 1563 OPPORTUNITIES TO MINIMISE ANIMAL USE IN REGULATORY TOXICOLOGY: A CROSS-COMPANY REVIEW.

S. Sparrow[1], S. Robinson[2], C. Bruce[3], A. Danks[4], D. Everett[5], R. Hill[6], H. Palmer[7] and K. Chapman[8]. *[1]GlaxoSmithKline, Ware, United Kingdom, [2]AstraZeneca, Macclesfield, Cheshire, United Kingdom, [3]Pfizer, Sandwich, Kent, United Kingdom, [4]Charles River, Tranent, Scotland, United Kingdom, [5]Covance Laboratories, Harrogate, United Kingdom, [6]Sequani, Ledbury, United Kingdom, [7]HLS, Huntingdon, United Kingdom and [8]NC3Rs, London, United Kingdom.*

Regulatory toxicology studies required for pharmaceutical development are well defined in terms of their scientific objectives. The UK Laboratory Animals Science Association and the National Centre for the Replacement, Refinement and

Reduction of Animals in Research established a group of toxicologists from the UK's major pharmaceutical companies and contract research organisations in order to share practice and identify areas for application of the 3Rs whilst ensuring that the scientific objectives and regulatory requirements of such studies are still met. A cross company review of numbers of animals used in general toxicology and carcinogenicity studies was carried out. The results showed there is some variation in the numbers of animals used. The reasons for this have been explored and the information used to develop a series of approaches where small changes in practice may reduce animal use. We recommend these approaches are used where possible but they will not be appropriate for all studies or programmes. Practical considerations are given on: reducing the number of animals to obtain toxicokinetic (TK) data; incorporating male fertility assessment into the six-month rodent toxicology study; including fewer recovery animals; and, using transgenic mice, single control groups and appropriate strains in carcinogenicity studies. The data collected demonstrate that the largest influence on animal numbers in rodent toxicity studies is for TK profiling. Therefore, the most significant contribution to reducing the number of animals is likely to be the development of analytical techniques which would allow analysis using smaller sample volumes.

PS 1564 THE LIMITED VALUE OF ACUTE TOXICITY TESTS IN SAFETY ASSESSMENT.

S. Robinson[1], S. Creton[2] and K. Chapman[2]. *[1]AstraZeneca, Macclesfield, Cheshire, United Kingdom and [2]NC3Rs, London, United Kingdom.*

A collaboration, led by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and AstraZeneca, analysed data from 70 compounds across therapeutic areas and demonstrated that acute toxicity studies had no value in risk assessment before the first clinical trials in humans1. In addition, consensus between clinicians, toxicologists, regulators and Poison Centres has been reached that acute toxicity studies are not used for managing overdose of pharmaceuticals and are of little value in treating human poisoning from chemicals2. Therefore, the last remaining driver for acute toxicity studies for pharmaceuticals has been removed. The impact of the pharmaceutical sector initiative has stimulated efforts to review the value of acute toxicity testing in other sectors. A working group, led by the NC3Rs, has highlighted circumstances where acute toxicity testing of non-pharmaceutical chemicals is redundant and may be avoided3. In addition, the European Partnership for Alternative Approaches to Animal Testing (EPAA), has established a multi-stakeholder team (including AstraZeneca, NC3Rs, ECVAM, the Humane Society and representatives of industry sectors) which has demonstrated that the primary regulatory driver for conducting acute toxicity studies across non-pharmaceutical sectors is classification and labelling 4. Further work into the value of acute toxicity studies for classification purposes is ongoing. These collaborations demonstrate the opportunities provided by creating a forum for a wide range of stakeholders to review whether animal toxicity studies are providing the data needed to make assessments of risk to human safety. The results will enable consensus to be reached on how to reduce the number of animals used and make the drug and chemical development process more efficient. 1 Robinson, S et al (2008). Regulatory Toxicology and Pharmacology, 50(3), 345-352
2 Chapman, K et al (2010). Regulatory Toxicology and Pharmacology, in press. 3 Creton, S et al (2010). Critical Reviews in Toxicology, 40: 50-83. 4 Seidle T, et al (2010) Toxicological Sciences, 116, 382-396

PS 1565 RISK ASSESSMENT OF ACETALDEHYDE (A TOBACCO SMOKE TOXICANT): INTEGRATION OF MARGIN OF EXPOSURE (MOE) AND MODE-OF-ACTION (MOA) EVALUATIONS.

F. H. Cunningham, S. Fiebelkorn and C. Meredith. *Group R&D, British American Tobacco, Southampton, United Kingdom.*

Identification of tobacco smoke toxicants for the purpose of product regulation has seen significant growth during recent years. Trends have been toward providing a quantitative risk estimate of the contribution of individual toxicants to disease with the aim of establishing priorities for risk reduction research. We previously proposed adoption of the Margins of Exposure (MOE) model, as part of a quantitative risk assessment paradigm. This model permits evaluation of both genotoxic and carcinogenic compounds and we propose its application to toxicants found in tobacco smoke. Our approach is to calculate MOE values from a range of published studies to determine consistency within available data sets. Computed MOEs enable segregation of toxicants into high and low priority groupings for risk reduction research depending on their relationship to the critical MOE value of 10,000. We suggest the integrated approach of using the MOE modeling technique in conjunction with the preparation of a mode of action (MOA) review. The example given here is for acetaldehyde where a proposed MOA consists of four key events eventually progressing to the development of tumours, coinciding with increasing exposure duration or dose. In line with the MOA, MOEs can be calculated for each of the identified key events: Cytotoxicity (Degeneration with hyper/metaplasia) = 1733 – 2007.; Genotoxicity = 1.9 – 896.; Hyper/Metaplasia = 771 – 1810.; Tumours = 165 – 1382. This integrated approach is the first step in combining risk assessment methodologies to produce a more physiologically relevant outcome. However, we also suggest that other tools, such as physiologically-based pharmacokinetic (PBPK) modelling, be used to ascertain a toxicants importance to smoking-related diseases. Further work should also investigate the options surrounding cumulative risk assessment, which may be of particular relevance to toxicants which share structural and biological activity patterns. This may begin to account for complex mixture exposure, as is the case for cigarette smoke.

PS 1566 CONTINUOUS INTRAVENOUS INFUSION STUDIES WITH IMPLANTED PORT CATHETER SYSTEMS IN FREE RANGING CYNOMOLGUS MONKEYS—A 20 YEAR EXPERIENCE REPORT.

S. H. Korte, P. Nowak, S. Friderichs-Gromoll, J. Kaspareit and E. Buse. *Covance Laboratories GmbH, Muenster, Germany.* Sponsor: G. Weinbauer.

Numerous pharmaceuticals and recently an increasing list of monoclonal antibodies undergoing regulatory pre-clinical trials in non human primates (NHP) are administered intravenously, often by continuous infusion. This overview summarizes experience from 447 Cynomolgus monkeys between 1989 and 2009, which underwent port-catheter system (PCS: PORT-A-CATH® and Covance patented port) implantation and dosing for up to 90 days. In case of multiple 24 hour daily infusions the maximum volume was 40 ml/kg, for once weekly infusions 60 ml/kg/24 h. The PCS was implanted with the catheter tip being inserted in the Vena cava caudalis. Dosing (CADD-Micro® or Pegasus® Vario pump) commenced following an adsorption test, jacket training, pump precision test and pre-study diagnostics. Haematology screening just prior to the start of dosing (following the PCS implantation plus a 2 week recovery period) confirmed the absence of haematological changes. A 10 IU/mL Heparin lock and a once weekly system flush ensured the free-flow of the PCS (dead space of 0.7 mL). Early problems such as (a) extraction of the catheter out of the vein, (b) port area necrosis and (c) needle loss, were overcome by (a) specialized training of animal handling, (b) better close body fixation of jacket and (c) advanced taping of the needle. No adverse clinical signs or body weight loss was noted. Microscopic findings post chronic catheterization were focally confirmed and included: perivascular haemorrhage, acute-chronic vasculitis/perivasculitis, hypertrophy/hyperplasia of the intima, perivascular edema, thrombi and organized thrombi at the injection site (tip of catheter). The same findings plus granulomatous inflammation (histiocytes and foreign body giant cells around suture material) occurred at the entrance of catheter into the vein. In summary, the presented work showed the long term feasibility of the implanted PCS system and the cumulative experience increases the likelihood of a successful infusion study in these primate species.

PS 1567 ENVIRONMENTAL PREDICTORS OF U.S. COUNTY MORTALITY PATTERNS ON A NATIONAL BASIS.

M. P. Chan[1], R. S. Weinhold[2], R. Thomas[3], J. M. Gohlke[4] and C. J. Portier[5]. *[1]NTP/NIEHS, Research Triangle Park, NC, [2]Independent Researcher & Journalist, Colorado City, Co., [3]School of Public Health, University of California, Berkeley, CA, [4]School of Public Health, University of Alabama at Birmingham, Birmingham, AL and [5]NCEH & ATSDR, CDC, Atlanta, GA.*

A growing body of evidence has found that mortality rates are positively correlated with social inequalities, air pollution, elevated ambient temperature, age, availability of medical care and other factors. This study develops a model that uses indicators for multiple factors to predict the mortality rates for selected diseases and life expectancy by county across the United States (US). The model is then applied to predict changes in mortality caused by changing environmental factors. A total of 3,110 counties in the US, excluding Alaska and Hawaii were studied. A subset of 519 counties from the 3,110 counties was chosen by using systematic random sampling and these samples were used to validate the model. Step-wise and linear regression analyses were used to estimate the linkage between environmental pollutants, socio-economic factors, risk factors, social capital, weather, crime, social and other factors to explain variations in county-specific mortality rates for cardiovascular diseases, cancers, chronic obstructive pulmonary disease, all causes combined and lifespan across five population density groups. Generally the estimated models fit adequately for all mortality outcomes for all population density groups. The model also adequately predicted risks for the 519 validation counties. Predictions of changing mortality with changing environmental pollutant factors are used to illustrate the predictive value of the model. This study confirms the complex inter-relationships of multiple factors that influence mortality and lifespan, and suggests the

need for a better understanding of the pathways through which these factors, mortality, and lifespan are related at the community level, particularly in urban versus rural settings. The resulting findings should provide policy makers at local, state, and federal levels with more explicit and feasible targets for policy intervention at the community level.

PS 1568 FIELD INVESTIGATION AND RISK ASSESSMENT OF FIVE CRUMB RUBBER ARTIFICIAL TURF FIELDS IN CONNECTICUT.

G. L. Ginsberg[1], B. Toal[1], N. Simcox[2], A. Bracker[2] and B. Golembiewski[3]. *[1]Environmental & Occupational Health, Connecticut Department of Public Health, Hartford, CT, [2]University of Connecticut Health Center, Farmington, CT and [3]Connecticut Department of Environmental Protection, Hartford, CT.*

Questions have been raised about health risks when playing on artificial turf fields cushioned with crumb rubber infill. Rubber is a mixture of volatile organic chemicals (VOCs), semi-volatile organic chemicals (SVOCs) and metals. Some components have toxic and carcinogenic properties. Exposure is possible, primarily via inhalation as chemicals emitted from rubber can reach the breathing zone and players have high ventilation rates. The Connecticut study involved laboratory offgas and field sampling and human health risk assessment. Air sampling was performed at 1 indoor and 4 outdoor artificial turf fields under summer conditions in Connecticut. On-field and background locations were sampled with stationary and personal samplers. A total of 27 chemicals of potential concern (COPCs) were found to be above background and possibly field-related. Lead was not elevated in the plastic grass or crumb rubber. COPCs were entered into separate risk assessments for outdoor and indoor fields and for children and adults. Inhalation rates were adjusted for play activity and for children's greater ventilation than adults. Toxicity values (cancer unit risks, RfCs, acute targets) were taken from national databases or derived by CTDPH. Field results indicated 10-20 times more rubber-related chemicals (naphthalenes, benzothiazole) in the indoor field compared to outdoors. Chronic cancer and non-cancer risks were not elevated indoors or out. The acute hazard index approached unity for children playing at the indoor field causing uncertainty about the potential for irritant effects indoors. In conclusion, this assessment did not find elevated health risks at the outdoor or indoor turf fields tested. However, it would be prudent for indoor field operators to provide adequate ventilation to prevent possible irritant effects from the buildup of rubber-related VOCs and SVOCs at indoor fields.

PS 1569 CRITICAL EVALUATION OF THE TOXICITY DATA FOR SELECTED PHTHALATE ESTERS.

K. R. Carlson, D. J. Williams, C. A. Osterhout and M. A. Babich. *U.S. Consumer Product Safety Commission, Bethesda, MD.*

Dialkyl phthalate esters (phthalates) are used as plasticizers in consumer and children's products made from polyvinyl chloride (PVC). Biomonitoring data show that virtually all Americans are exposed to phthalates on a daily basis. The relative contributions of different sources (i.e., food, water, air, dust, consumer products) of phthalate exposures are not clear. The authors reviewed all available toxicity data for six phthalate esters (butylbenzyl [BBP], dibutyl [DBP], di(2-ethylhexyl) [DEHP], diisononyl [DINP], diisodecyl [DIDP], and di-n-octyl [DnOP]) as the first step of a risk assessment. For each phthalate, acceptable daily intake (ADI) values were calculated for all chronic toxicity endpoints resulting from oral exposures, provided that sufficient data were available. Several phthalates were found to adversely affect multiple organ and physiological systems including development and reproduction. The lowest oral ADIs (effect in parentheses) calculated for each phthalate were: 0.0058 mg/kg-d (DEHP; testes), 0.12 mg/kg-d (DINP; liver), 0.15 mg/kg-d (DIDP; liver), 0.2 mg/kg-d (DBP; reproductive), 0.37 mg/kg-d (DnOP; liver), and 1.0 mg/kg-d (BBP; liver). ADIs for developmental toxicity endpoints were: 0.011 mg/kg-d (DEHP), 0.4 mg/kg-d (DIDP), 0.5 mg/kg-d (DBP), 1.0 mg/kg-d (DINP), and 0.7 mg/kg-d (BBP). There were insufficient data to derive a developmental ADI for DnOP. ADIs were within the ranges of ADIs derived by other organizations. Summary reports and ADIs for each phthalate were peer-reviewed by independent experts. These ADIs can be used with exposure estimates to assess human health risks from phthalates in consumer products. These materials were prepared to assist a Chronic Hazard Advisory Panel (CHAP) convened by the CPSC "to study the effects on children's health of all phthalates and phthalate alternatives as used in children's toys and child care articles" (section 108 of the Consumer Product Safety Improvement Act of 2008). *This work has not been reviewed or approved by, and does not necessarily represent the views of, the Commission.

PS 1570 TOXICITY OF PARTICULATE MATTER FROM AFGHANISTAN AND IRAQ IN A TWO-WEEK INHALATION STUDY IN RATS.

B. A. Wong[1, 2], K. C. Roberts[2], C. U. Parkinson[2], V. P. Mokashi[1] and P. A. Ortiz[1]. *[1]Naval Medical Research Unit - Dayton, Wright-Patterson AFB, OH and [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Personnel deployed in the Middle East may be exposed to particulate matter (PM) from the surrounding environment. Some *in vitro* cell culture studies have indicated differences in the potential toxicity of PM from various deployment locations in the Middle East. PM from Iraq (Talil), as compared with PM from other locations (Camp Victory), showed increased lactate dehydrogenase (LDH) and decreased production of MTT, indicators of cellular toxicity. To investigate this difference in an *in vivo* model, an aerosol of PM from Afghanistan or Iraq was generated and introduced into a whole body inhalation chamber. Sprague-Dawley rats were exposed to Afghan or Iraq PM at 1 mg/m^3 or clean air for 20 h/d, 7 d/wk for 2 weeks. After the last exposure, one set of animals from each exposure group was necropsied immediately and one set was held in clean air for a 3 week recovery period. At necropsy, blood was taken for clinical chemistry and hematology, and bronchial alveolar lavage (BAL) fluid was collected. Animals did not show significant differences in group average body weights for the different PM. There were statistically significant differences in clinical chemistry and hematology results; however, there was no overall pattern to indicate organ damage or other biochemical dysfunction. In addition, there was no significant difference in the LDH or total protein concentrations in BAL fluid. Concentrations of inflammatory cytokines, MIP-1α, IL-1β, IL-6, and TNF-α measured in BAL fluid were below limits of detection. Finally, histopathology did not identify any PM in the lungs and there were no findings that were considered to be induced by the test material. While *in vitro* studies identified PM with potential toxicity, *in vivo* inhalation studies showed no toxicity under the conditions of this study.

PS 1571 TOXICITY OF EXCITATORY AMINO ACIDS IN RED ALGAE FOR PARASITIC COPEPODES OF CULTURED PUFFERFISH.

K. Yasui[1] and M. Asakawa[2]. *[1]Graduate School of Biosphere Science, Hiroshima University, Hiroshima Prefecture, Hiroshima Prefecture, Japan and [2]Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Hiroshima Prefecture, Japan.*

The tiger puffer Takifugu rubripes is an economically important fish, and is extensively cultured in western Japan. In aquaculture industry, caligid copepods are well known as pests causing heavy mortality or acting as disease inducers by creating a portal for entry of bacteria or other pathogens. The caligid *Pseudocaligus fugu* is a common ectoparasite of marine puffer fishes in Japan. Our a preliminary data show that crude H_2O extract of "kaininso", or a red alga *Digenea simplex* showed effects on the parasite. The behavior of *P.fugu* was observed under microscope to determine its activity.The parasitic copepods clearly ceased swimming behaviors within 1 hr after it was immersed. Attempts were made to isolate the active substances from specimens of a red alga, *D. simplex* (wet weight 430 g) from Ishigaki Island, Okinawa Prefecture, in June, 2009. They were extracted with distilled water overnight, and centrifuged. The supernatant through a Spectra/Por 3 dialysis membrane, was added to the activated charcoal, and active component adsorbed were eluted with acidic methanol. The eluate was chromatographed on a ODS prepacked column with 1% acetic acid in 10% aqueous acetonitrile as a mobile phase. The fraction thus obtained were concentrated and chromatographed on Bio-Gel P-2 column with 0.03M acetic acid. Fractions of 5 mL were collected at a flow rate of 1.0 mL/min. Each fraction was analyzed by high-performance liquid chromatography (HPLC) using a Lichrospher 100 RP-18(e) column (Merck) with 0.1% trifluoroacetic acid in 10% aqueous acetonitrile as a mobile phase. Fractions from the column were monitored at 242 nm. A major active substance to the parasitic copepodes of cultured pufferfish found in fractions 12-19 were identified as kainic acid from the results of TLC, LC-MS and NMR spectral analysis.

PS 1572 RESULTS OF A ONE-YEAR FISH CONSUMPTION SURVEY IN ALABAMA.

E. S. Ebert[1], N. Wilson[2], M. Wacksman[1], A. Fowler[3], J. Schell[4] and J. Loper[5]. *[1]ARCADIS, Portland, ME, [2]ARCADIS, Brighton, MI, [3]ARCADIS, Beverly, MA, [4]Entrix, Houston, TX and [5]The Loper Group, Seabrook, TX.*

To support a human health risk assessment for Choccolocco Creek in Alabama, a one-year intercept survey was conducted. The survey collected information about the extent of usage, locations fished, species and sizes of fish harvested for consumption, methods used to prepare and cook them, rates at which they were consumed, and demographic characteristics of the user population. The survey was

conducted on 101 survey days, representing 27 percent of available days during the one-year period. The sampling days were selected based on a stratified random sample method that accounted for season, time of week, location and time of day. In total, 72 anglers were observed during the one-year survey period and 52 of those individuals completed interviews. After correcting for avidity bias, it was estimated that a total population of 173 anglers fish the creek each year. The interviewed anglers fished an average of four months during the year and took an average of two fishing trips during a typical four-week period. Estimated trips per year ranged from 1 to 54 with an average rate of seven trips per year. Based on the interviews conducted, anglers were successful at harvesting fish for consumption on 15 percent of the fishing trips taken and only three of the 52 anglers interviewed at the end of their trip had harvested fish for consumption. Consumption rates were estimated for each angler who had harvested fish based on the mass of fish harvested by trip end, an edible portion of 30 percent, the reported frequency of fishing trips, the average success rate, and the number of persons to share in consumption. The estimated annualized average fish consumption rates ranged from 0.14 g/day to 7.9 g/day with a mean rate of 2.9 g/day. Currently, there is a fish consumption advisory for the creek. However, comparison of these rates with rates reported for a similar small water body in Alabama, which had no advisory at the time of the survey, indicates that suppression is not occurring.

PS 1573 ANALYSIS OF TOP 10 VOLATILE ORGANIC COMPOUNDS (VOCS) TO EVALUATE THE INDOOR AIR QUALITY WHICH MAY CAUSE SICK BUILDING SYNDROME.

M. Hanazato[1, 2], E. Todaka[3, 4], H. Nakaoka[1, 4] and C. Mori[1, 3, 4]. *[1]Center for Preventive Medical Science, Chiba University, Chiba, Japan, [2]Department of Architecture, Graduate School of Engineering, Chiba University, Chiba, Japan, [3]Center for Environment, Health and Field Sciences, Chiba University, Kashiwa, Japan and [4]Department of Bioenvironmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan.*

"Sick Building Syndrome (SBS)" is a series of symptoms such as headache, eye irritation, throat ache, caused in newly built or remodeled buildings. The major causes of SBS are suspected to be volatile organic compounds (VOCs) indoor air. To solve this problem fundamentally, a model town called "Chemiless Town" was constructed in the university campus. The causing chemicals are different among individuals, however, higher the levels of the VOCs, more people show the symptoms. To find the causing chemicals, 116 compounds (63 VOCs, 42 SVOCs and 11 aldehydes) indoor air of the 6 rooms of the 3 Experimental Houses (EH) in Chemiless Town were analyzed and calculated from April 2007 to May 2009. The total of 116 VOCs (TVOCs) of EH A were 520μg/m^3 in April 2007, 225μg/m^3 in April 2008, 37μg/m^3 in May 2009. The TVOCs of EH B were 1,178μg/m^3 in April 2007, 560μg/m^3 in April 2008, 133μg/m^3 in May 2009.The TVOCs of EH C were 14,776μg/m^3 in April 2007,1,046μg/m^3 in April 2008, 306μg/m^3 in May 2009. The TVOC of EH A was the lowest among the 3 houses. The highest TVOC 17,569μg/m^3 was found in EH C in April 2007. The concentration levels of each VOC were extremely variable depending on the materials of the houses and the seasons. However, it was suggested that it was possible to evaluate the indoor air by calculating top 10 VOCs indoor air. In EH A, in which the TVOC was the lowest among the 3 EHs, the rate of concentration of the top 10 VOCs ranged from 52 to 100%. In EH C, in which the TVOC was the highest, it ranged from 83 to 93%, and most of them was terpens, especially α-Pinen. It became clear that it was possible to evaluate the indoor air VOCs if only top 10 or fewer VOCs were analyzed.

PS 1574 EFFECT OF FEED RESTRICTION ON ROUTINE TOXICITY PARAMETERS IN WISTAR RATS.

S. Jana, R. Nirogi, M. A. Mulla, S. Pandey, V. Goyal, A. Gothi, R. Vedamurthy and R. Mishra. *Toxicology, Suven Life Sciences Limited, Hyderabad, Andhra Pradesh, India.* Sponsor: V. Reddy.

In preclinical safety studies, changes observed in different toxicological parameters are often difficult to interpret in relation to primary compound effects in presence of reduced feed consumption. Hence the present study was undertaken to study the effects of feed restriction on different routinely evaluated toxicological parameters in Wistar rats. Five groups of rats (10/sex/group) were fed ad libitum (control), or given 90% (mildly restricted), 75% (moderately restricted), 50% (markedly restricted), or 25% (severely restricted) of the amount of feed consumed the day before by control rats. One set of 5 rats from each group was sacrificed after 7 days and other set was sacrificed after 14 days of feed restriction. Different parameters were studied during experiment period and at termination. All rats survived throughout the experiment period except one female from severely restricted group died on day 11. Clinical signs like emaciation, excitation and rough hair coat were observed in marked and severely restricted groups. Terminal body weights of mildly and moderately restricted groups were lower than the control rats. The moderately restricted group males did not grow whereas females from moderately restricted group and both males and females from severely restricted group lost body weight throughout the study. There was decreases in water consumption and urine volume in feed restricted groups. Hemoconcentration and changes in different clinical chemistry parameters were observed in severely restricted group. Further, absolute weights of routinely weighed organs in severely restricted group were reduced and effect was proportional to effect on body weight. Microscopically atrophic changes were observed in various parenchymatous organs in markedly or severely restricted groups and the severity of the changes was increased with the duration of feed restriction. In conclusion, severe and marked decrease in feed consumption can alter various toxicological parameters, which should be considered during the safety evaluation of different compounds.

PS 1575 ACUTE NEUROBEHAVIORAL EFFECTS OF INHALATION EXPOSURE TO ETHYL ACETATE - A PILOT STUDY IN HUMAN VOLUNTEERS USING EVENT-RELATED POTENTIALS.

S. A. Juran[1, 2], S. Kleinbeck[2], M. Schäper[2], G. Johanson[1] and C. van Thriel[2]. *[1]Work Environment Toxicology, Karolinska Institutet, Stockholm, Sweden and [2]Unit for Neurobehavioral Toxicology and Chemosensation, Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, Germany.*

Exposure to neurotoxic compounds results in reduced efficiency of the central nervous system and performance deficits in cognitive domains which can be measured by neurobehavioral methods. We extended common behavioral tests including reaction times (RT) and amount of errors (E) by adding electroencephalographic measurements of event-related potentials (ERP). Six male volunteers were whole-body exposed to ethyl acetate during 4 h at three conditions: 2 ppm, 400 ppm, and 0-800 ppm including exposure peaks. The 2 ppm level was used as odorous control condition, while the two higher levels correspond to the German OEL. After 5, 90 and 180 minutes of exposure, the subjects performed cognitive tasks that required the inhibition of motor responses and that were potent to evoke erroneous behavior. We assume response inhibition to be a sensitive function for ETA effects because it has shown sensitivity for ethanol effects, one of the ETA metabolites. RT and E were examined at the behavioral level. ERP components representing motor-inhibition and error-processing were analyzed at the cortical processing level. The study was approved by the local ethic committee of the IfADo and sponsored by The German statutory accident insurance (DGUV). None of the behavioral variables were affected in a dose-dependent manner. Visual inspection of the ERP data in the cross-subject grand averages suggested concentration-dependent effects on components representing response inhibition and error processing. However, these effects were not statistically significant, probably due to the small sample size. Overall, our attempt is promising and electrophysiological methods should be further evaluated for assessment of acute neurobehavioral effects of chemicals.

PS 1576 CASE STUDY: RISK ASSESSMENT WITH A MULTI-KINASE INHIBITOR WITH ONCOLOGICAL INDICATION.

M. Brughera, R. Pulci, P. Colombo and A. Giusti. *Preclinical Development, Accelera Srl, Nerviano - Milano, Italy.*

A variety of new chemical entities, able to inhibit multiple receptor tyrosine kinases and their downstream pathways, have recently emerged as oncological drugs. We report here the case study of a new small molecule acting as a multikinase inhibitor with promising antitumor activity. This compound has been extensively tested in pharmacodynamic, pharmacokinetic and toxicology studies. Preclinical safety studies conducted in rats and dogs have shown that the major target organs affected under repeated administration were the hemolymphopoietic (HLP) system and liver in both species, followed by CNS effects occurring at higher exposure levels. CNS clinical signs (lack of coordination, staggering, depression) were observed after 6-7 days of repeated administration of high doses in rats and dogs, but recovered within one week after drug withdrawal. In dogs, CNS signs were also noted at lower doses/exposures but only after longer treatment periods (≥ 2 weeks). These effects were not associated with morphological changes in the brain but were consistent with crossing of the blood brain barrier. The brain to plasma ratios were higher in dogs than in rats, confirming the relative sensitivity of dogs as far as the CNS effects were concerned. The involvement of TrkA, one of the kinases targeted and significantly over-expressed in the brain, can be postulated. A further effect observed only in dogs was a moderate prolongation of the QT interval occurring at high doses/exposures in those animals previously showing severe CNS effects. To investigate the time/dose-dependent correlation of the CNS clinical signs and QT prolongation with systemic exposure, a time-course study was conducted in dogs. This study demonstrated strict correlations of the CNS effects and the QT prolongation with systemic exposure (reaching specific thresholds) and treatment duration. Higher therapeutic indices could be obtained by adapting the schedule of administration. In view of the superior efficacy and intended population the risk assessment was favorable to conduct clinical trials.

PS 1577 AN *IN VITRO* MODEL SYSTEM FOR ASSESSING THE EFFECTS OF OIL DISPERSANTS IN HUMAN HEPATOCYTES.

O. Bandele, M. Santillo and P. Wiesenfeld. *Toxicology, Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, U.S. FDA, Laurel, MD.*

Because of the unprecedented amount of oil dispersants utilized during the 2010 Deepwater Horizon oil spill, understanding the toxicity of these chemicals is crucial. These dispersants may accumulate in aquatic species, be passed down the food chain, where if consumed in significant quantities may have a negative affect on human health. The majority of ingested chemicals, drugs, pollutants, and toxins are metabolized by the liver. We therefore examined the effects of several oil dispersants on a human liver cell line, HepG2/C3A, which serves as an in vitro cell model. We employed spectrofluorimetry-based endpoint assays to assess the toxicity of 10-500 ppm EC9527A, EC9500A, and ZI-400 in HepG2/C3A cells. Significant increases in cell death were observed after exposing cells to EC9527A and EC9500A for a maximum of 72 hr (LC50 ~325 ppm), whereas ZI-400 was moderately cytotoxic (LC50 > 400 ppm). Endpoint assays that monitored reactive oxygen species demonstrated that all dispersants caused a dose-dependent accumulation of reactive oxygen species in HepG2/C3A cells. We also observed that EC9500A and ZI-400 disrupted the mitochondrial membrane electrical potential, monitored by the uptake of rhodamine 123. In addition, EC9527A may inhibit P-glycoprotein activity. Our initial findings suggest that the dispersants examined in this study may act through different mechanisms. These results also demonstrate an effective in vitro approach to identify and evaluate dispersants and related chemicals that may be potential hepatotoxins.

PS 1578 CHARACTERIZATION OF IONIC LIQUID COMPOUNDS FOR TOXICOLOGICAL EVALUATION.

J. W. Algaier[1], C. C. Pearson[1], A. D. Ammenhauser[1], Q. Lawrence[1], J. L. Cookinham[1], L. G. Siemann[1], R. K. Harris[1], B. Jayaram[2] and C. S. Smith[2]. *[1]Midwest Research Institute, Kansas City, MO and [2]NIH, NIEHS, Research Triangle Park, NC.*

Ionic liquid (IL) compounds are a new class of chemicals replacing regular organic solvents and are used in diverse reactions under 'green chemistry'. The structural flexibility of IL's and the varied array of available anions have made it possible to tune solvents for a specific reaction. However, little is known about the toxicity of these compounds and for this reason four ionic liquids have been selected for toxicological evaluation by the NIEHS, National Toxicology Program (NTP), which are 1-Butyl-3-methyl-imidazolium chloride, 1-Butyl-1-methyl-pyrrolidinium chloride, 1-ethyl-3-methylimidazolium chloride, and N-Butyl-pyridinium chloride. For this work, identity and impurity determination used several methods including direct infusion liquid chromatography/mass spectrometry (LC/MS), where parent and daughter ion scan spectra confirmed identity. The infrared (FT-IR) and nuclear magnetic resonance (NMR) spectra are consistent with the literature structures. Because of the hydrophobic nature of ionic liquid compounds, special handling procedures using inert argon atmosphere were required, especially to determine the water content by Karl Fischer, which averaged 0.36%. The volatile content by weight loss on drying was attempted, but only successful with one compound at 0.75% since a constant weight loss was not achieved. Chloride content was quantitated using ion chromatography and elemental analysis for carbon, hydrogen, nitrogen and chloride was performed; results from both methods matched theoretical values within +/- 0.5%. The HPLC purity profile analysis using an isocratic acetonitrile-ammonium acetate mobile phase with a cyano column and ultraviolet (UV) and evaporative light scattering detection (ELSD) confirmed no observed impurities under these conditions. The results confirmed the identity of each ionic liquid compound with purity values greater than 99%.

PS 1579 ANALYSIS OF ACUTE AND CHRONIC EFFECTS OF HETEROCYCLIC AROMATIC AMINES IN DIFFERENTIATED HUMAN HEPATOMA HEPARG CELLS.

J. Dumont[1, 2], R. Josse[1, 2], C. Lambert[1, 2], S. Anthérieu[1, 2], C. Guguen-Guillouzo[1, 2], A. Guillouzo[1, 2] and M. Robin[1, 2]. *[1]UMR991, INSERM, Rennes, France and [2]University of Rennes 1, Rennes, France.* Sponsor: M. Pallardy.

Humans are continuously exposed to low doses of various food and environmental contaminants. Prediction of their toxicity and risk assessment for human health represent currently a major challenge. We have compared the effects of acute and repeat exposure to two common heterocyclic aromatic amines (HAA), 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), singly or in equimolar mixture, in differentiated human hepatoma HepaRG cells using various experimental approaches, including the comet assay to assess DNA damage and transcriptomic technologies. After a 24 h-exposure, PhIP and MeIQx, singly and in mixture, exerted differential effects on apoptosis, oxidative stress, DNA and cytochrome P450 (CYP) activities. Only PhIP caused DNA damage. PhIP was also a stronger inducer of CYP1A1 and CYP1B1 expression and activity than MeIQx. In contrast, only MeIQx significantly induced CYP1A2 activity. The combination of PhIP with MeIQx caused an oxidative stress and showed synergistic effects on apoptosis but inhibitory effects on DNA damage. In addition, we analyzed gene expression profiles after either a single 24 h- or a repeat 28 day-exposure to PhIP or MeIQx. The most responsive genes to both HAA were downstream targets of the arylhydrocarbon receptor: CYP1A1 and CYP1A2 after both time points and CYP1B1 and ALDH3A1 after 28 days. Several other genes, mainly related to cell growth, apoptosis and cancer, exhibited to a lesser extent both time-dependent and compound-specific expression changes. Our findings highlight the need to investigate both acute and chronic effects of contaminants, singly and in mixture, as well as their interactions when assessing risk for human health and demonstrate the unique properties of HepaRG cells as a metabolically competent cell line for chemical toxicity testing. (Dumont et al. TAAP. 2010, 245:256 and Dumont et al. TAAP in press. Financial support: ANR, contract 06SEST17).

PS 1580 THE MAXIMUM CUMULATIVE RATIO (MCR): A TOOL FOR ASSESSING THE NEED FOR CUMULATIVE RISK ASSESSMENTS.

X. Han and P. S. Price. *Toxicology & Environmental Research & Consulting, The Dow Chemical Company, Midland, MI.*

Cumulative risk assessments are resource intensive. Maximum cumulative ratio (MCR) is a tool that identifies chemicals where cumulative risk assessments are most necessary. The MCR is the ratio of the total risk of a group of chemicals to the largest single-chemical risk. Smaller values of MCR imply less need for risk assessments. Two case studies of the approach are presented. Methods: Biomonitoring data were taken from NHANES, for serum levels of 26 dioxins, furans, and PCBs (D/F/Ps) and urine levels of 6 phytoestrogens and 3 estrogenic phenols (PEs). The cumulative toxicity of the levels of the chemicals was evaluated using toxicity equivalency factors (TEFs). WHO and Haws et al. TEFs were used for D/F/Ps and TEFs for PEs were developed from published toxicity data. MCR values were determined and plotted against individuals' total body burden. Simulation models were used to investigate the impact of uncertainty in correlations, uncertainty in the TEFs, and adding more chemicals to the assessment. Results: MCR values for individuals were found to range from 1-5 for D/F/Ps and 1-3.5 for PEs. Mean values for PEs (1.7) were lower than D/F/Ps (3.3). Values decreased with higher body burdens for PEs and for D/F/Ps when TEFs from Haws et al. were used but increased slightly when the WHO TEFs were used for the D/F/Ps. Simulations show that increasing the number of chemicals had a moderate effect and increasing inter-chemical correlations increased values by ~1.5. Conclusions: In both cases the majority of toxicity came from a small fraction of chemicals, the D/F/Ps were dominated by 4-5 of the 26 chemicals while the PEs were dominated by 1-2 of the 9 chemicals. The MCR values decreased with total risk for PEs but not for D/F/Ps. These findings suggest that there is more need for a cumulative risk assessment for D/F/Ps than for PEs and demonstrate the ability of the MCR to differentiate between mixtures that do, and do not, warrant cumulative risk assessments.

PS 1581 EVALUATION OF THE POTENTIAL IMPACT OF INHIBITION OF TRICHLOROETHYLENE METABOLISM IN THE LIVER ON EXTRAHEPATIC TOXICITY.

C. R. Eklund, M. V. Evans and J. Simmons. *NHEERL, U.S. EPA, Research Triangle Park, NC.*

The interaction between trichloroethylene (TCE) and chloroform (CHCl3) is less than additive, with co-exposure to TCE and CHCl3 resulting in less hepatic and renal toxicity than observed with CHCl3 alone. Vapor uptake data demonstrate that co-exposure to CHCl3 decreases the rate of disappearance of TCE from the vapor uptake chamber. Physiologically-based pharmacokinetic (PBPK) modeling of vapor uptake data for TCE alone, CHCl3 alone, and binary mixtures of TCE and CHCl3 indicate that competitive inhibition best describes the effect of CHCl3 on TCE metabolism. We have previously determined that the acute neurotoxic effects of TCE are predicted on the basis of the momentary concentration of TCE in either the blood or the brain. Therefore, the purpose of the present study was to understand the impact of decreased TCE metabolism in the presence of CHCl3 on the concentration of TCE in the blood. The vapor-uptake, five-compartment (blood, liver, fat, slowly and rapidly perfused) PBPK model was used to estimate the concentration of TCE in blood at the end of a 10 min, 1 hr or 8 hr exposure to initial starting concentrations of either 500 ppm TCE alone, 500 ppm TCE in combination with 500 ppm CHCl3, 1000 ppm TCE alone and 1000 ppm TCE in combination with 1000 pm CHCl3. At both 500 and 1000 ppm TCE, concurrent exposure to CHCl3 resulted in small increases in blood TCE concentrations at all three

time intervals. The percentage increase ranged from ~5% to ~15% with the largest increases seen at 500 ppm TCE with 500 ppm CHCl3. These model simulations indicate that increased blood concentrations of TCE (and increased neurotoxicity) may result from co-exposure to CHCl3. Similar simulations conducted with our human model (currently under development) will allow us to evaluate the degree of increased risk at low concentrations typically seen in the environment and at high concentrations that might result from accidental or intentional chemical releases. (This abstract does not reflect EPA policy.)

PS 1582 A COMPUTATIONAL FRAMEWORK FOR CUMULATIVE RISK ASSESSMENT.

D. A. Sarigiannis[1, 2], A. Gotti[3] and S. Karakitsios[1]. [1]*Institute for Health and Consumer Protection, European Commission - Joint Research Centre, Ispra, Varese, Italy,* [2]*Chemical Engineering Department Aristotle University of Thessaloniki, Thessaloniki, Greece and* [3]*CPERI, CERTH, Thermi, Greece.* Sponsor: T. Hartung.

The complexity in quantitatively estimating human exposure to environmental chemicals originates from the need to assess their aggregate and cumulative exposure; the assessment becomes even more complex when mixture effects need to be taken into account. In this study a framework for quantitative cumulative assessment of the biologically effective dose of several interacting environmental chemicals is developed in order to better take into account biomarkers of exposure towards compiling the exposome. The integrated modelling environment was implemented in a single computational platform (asclXtreme) including emissions, dispersion, exposure modelling, internal dose and health risk. A central novel component of the platform is a generic Physiology Based ToxicoKinetic/Dynamic (PBTK/D) model, coupled to a Biology Based Dose Response (BBDR) model. Uncertainty and variability of the affecting parameters were estimated through Marcov Chain Monte Carlo. Risk from co-exposure to benzene, toluene, ethylbenzene and xylenes (BTEX) were selected as case study under different environmental exposure scenarios. The methodology permits the estimation of health risks capturing the continuously changing environmental and biological dynamics. The integrated modeling platform was tested in several BTEX exposure scenarios (occupational and environmental). The estimation of both cancer and neurotoxicity risk due to benzene and toluene was much refined when the internal dose of the parent substance and its metabolites rather than only personal exposure linked to epidemiological relations was taken into account. This is due to two reasons: (a) Aggregation of exposure through different microenvironments with continuously changing concentrations results in differences in the daily variation time profile of external exposure and internal dose; (b) The presence of co-exposure to the other VOCs in the mixture affects the levels of benzene metabolites through inhibition of benzene metabolism.

PS 1583 USE OF A CUSTOM RT-PCR ARRAY TO ANALYZE TOXICITY PATHWAYS AT DIFFERENT LIFE STAGES IN BROWN NORWAY RAT BRAIN FOLLOWING ACUTE TOLUENE EXPOSURE.

J. E. Royland[1], P. R. Kodavanti[2], G. W. Knapp[1] and R. C. MacPhail[2]. [1]*Integrated Systems Toxicology Division U.S. EPA, Research Triangle Park, NC and* [2]*Toxicity Assessment Division, U.S. EPA, Research Triangle Park, NC.*

To investigate the contribution of different life stages on response to toxicants, we utilized a custom designed RT-PCR array to examine the effects of acute oral exposure of the volatile organic solvent toluene (0, 0.65 or 1.0 g/kg) in the brains of male Brown Norway rats at 4, 12 and 24 months of age. Toluene is a known neurotoxicant with effects on cognition, motor activity and neurotransmitters in the central nervous system. The objective was to explore the toxicity pathways that contribute to the adverse effects of toluene exposure, and to determine if the response is age-dependent. In this study, we examined gene expression in cerebellum, striatum, hippocampus and frontal cortex 4 hours post-dosing using a custom RT-PCR array containing 30 genes representing key toxicity pathways involving energy metabolism, oxidative stress, neuronal plasticity, immune and stress responses. Results showed that the effect of age exceeded that of toluene on the number of genes affected (approx 2 to 5x depending on brain area). With the exception of the toluene effect in striatum, percent of up regulation (averaged ~80% with age and near 100% with toluene) exceeded that of down regulation in this subset of genes selected as possible markers of susceptibility. Based on number of genes affected, cerebellum displayed the greatest sensitivity. However, based on direction of expression changes, striatum was most vulnerable. These data indicate that response to toxicant exposure is impacted by life stage, which, in itself, affects gene expression levels. Data also indicate variability in response across brain areas, with degree and direction of change depending upon condition tested. These ongoing studies contribute to defining a genomic model of toxicity pathways in the nervous system and their modification with aging. (This abstract does not necessarily reflect U.S. EPA policy).

PS 1584 COMPARATIVE ANALYSIS OF TCDD-ELICITED GENE EXPRESSION PROFILES IN HUMAN, MOUSE, AND RAT PRIMARY HEPATOCYTES.

E. Dere, A. L. Forgacs and T. R. Zacharewski. *Michigan State University, East Lansing, MI.*

TCDD is a ubiquitous environmental contaminant that induces species-specific effects. A comparative approach was utilized to examine TCDD-elicited time- and dose-dependent differential hepatic gene expression in human, mouse, and rat primary hepatocytes isolated from immature Sprague-Dawley rats, CD-1 mice and 3 post-menopausal, non-obese, non-smoking Caucasian donors, respectively. Cells were treated with 10 nM TCDD or DMSO vehicle control for 1, 2, 4, 8, 12, 24 and 48 h, or with 0.001, 0.01, 0.1, 1, 10 or 100 nM TCDD for 12 and 24 h. Whole-genome microarrays identified 2423, 763 and 541 differentially expressed genes across time in human, mouse, and rat hepatocytes, respectively (P1(t)>0.9 and |fold change|>1.5). Only 17 orthologs were responsive to TCDD in all three species, including Cyp1a1, Cyp1a2 and Tiparp. Cyp1a1 exhibited dose-dependent induction in all species with EC50 values of 0.04 nM for human, 0.05 nM for mouse, and 0.02 nM for rat hepatocytes at 12 h. However, commonly regulated genes across all species exhibited divergent expression profiles. For example, Bcl2-like 11, apoptosis facilitator (Bcl2l11) was induced by TCDD in the mouse and rat ~2-4-fold but repressed ~4-fold in the human hepatocytes. Meanwhile, 2097, 533 and 400 genes were unique to the mouse, rat and human, respectively, demonstrating that the majority of TCDD-elicited changes were species-specific. Functional annotation of differentially regulated genes identified processes related to lipid metabolism, molecular transport and cellular growth, and proliferation in all three species, suggesting conserved modes of action but different mechanisms of action. Thus, although the AhR and its signaling mechanism is highly conserved, species-specific gene expression profiles likely determine the species-specific effects of TCDD. Funded by SBRP P42ES04911.

PS 1585 DESCRIPTIVE 'OMICS OF ACETAMINOPHEN IN HUMANS.

M. Jetten[1], S. Gaj[1], A. Ruiz-Aracama[2], T. de Kok[1], J. van Delft[1], S. Claessen[1], A. Lommen[2], A. Peijnenburg[2], L. Pellis[3], E. van Someren[3], R. Stierum[3] and J. Kleinjans[1]. [1]*Health Risk Analysis & Toxicology, Maastricht University, Maastricht, Netherlands,* [2]*Institute of Food Safety, RIKILT, Wageningen, Netherlands and* [3]*Quality of Life, TNO, Zeist, Netherlands.* Sponsor: H. van Loveren.

Genomics and metabolomics, so called 'omics technologies, have undergone major improvements over the last decade. As a result, their applicability to human observational studies has become feasible over the recent past. The present study aims at investigating the capability of transcriptomic and metabolomic techniques to measure low dose effects of the well-investigated hepatotoxic compound acetaminophen (paracetamol or APAP) in blood/urine from healthy individuals. Both metabolomic (NMR/UPLC-TOF-MS) and genomic (mRNA/miRNA arrays) technologies were applied to a study on blood and urine from 6 healthy volunteers exposed to 0.5, 2 and 4 gram APAP/24 hours. Blood was collected after 0, 1, 7 and 24 hours of APAP exposure and urine was collected both before and during the APAP exposure window. The results showed the hepatotoxic phase I metabolites of APAP (NAPQI-bound metabolites) to increase with APAP dose. Also, several novel APAP metabolites were identified. Transcriptomics analysis at the mRNA level indicated immune and toxicity related pathways to be affected after 2 and/or 4 gram APAP/24 hour exposure. miRNAs also indicated that similar pathways were affected. At the lowest dose of 0.5 gram APAP/24 hours, the limit of detection of 'omics analysis in blood/urine from humans appears to be reached. Classic clinical test were not able to detect responses indicative for liver toxicity in blood at any of the exposure doses. From this we conclude that in 'omic techniques are able to detect exposure to low APAP doses and have the power of revealing a wealth of biological information. Even though only therapeutic doses were used and there were no signs of overt liver toxicity, effects related to both efficacy and toxicity were measured, supporting the applicability of 'omics as an observational tool for human studies.

PS 1586 CISPLATIN-INDUCED GENOTOXIC STRESS IN PRIMARY MOUSE HEPATOCYTES, MOUSE EMBRYONIC STEM CELLS, AND HEPG2 CELLS.

L. Rieswijk[1, 2], D. Lizárraga[1, 2], K. Brauers[1], B. van de Water[2, 3], H. Vrieling[2, 3], J. van Delft[1, 2] and J. Kleinjans[1, 2]. [1]*Health Risk Analysis and Toxicology, Maastricht University, Maastricht, Netherlands,* [2]*Netherlands Toxicogenomics Centre, Maastricht, Netherlands and* [3]*Toxicogenetics, LUMC, Leiden, Netherlands.* Sponsor: H. van Loveren.

By focusing on relatively new fields of toxicogenomics, such as epigenetics and microRNA (miRNA), this current NTC project tries to reveal novel mechanisms by which genotoxic and non-genotoxic compounds could act. MiRNAs regulate

gene expression post-transcriptionally and are expected to play an important role in xenobiotic responses. We used the genotoxic anticancer drug cisplatin (CDDP) as a test compound in several in vitro models to characterize and compare their genotoxic response patterns in relation to induced miRNA profiles. Primary mouse hepatocytes, mouse embryonic stem cells (mESC) and the hepatoma cell line HepG2 are frequently used in vitro models for genotoxicity studies. Cell viability, DNA damage, apoptosis, cell cycle, gene expression and miRNA profiles were investigated in these models following exposure to different concentrations of CDDP measured at several time points. DNA damage could be detected reliably with the yH2AX staining assay in both the primary mouse hepatocytes and in HepG2 cells. CDDP caused DNA damage in both cell lines. Flow cytometric analysis in HepG2 cells and mESC revealed that damaged cells were blocked in the S-phase and underwent apoptosis. Analysis of the gene expression data in MetaCore confirmed that pathways involved in "DNA damage", "apoptosis" and "cell cycle" were mainly affected in HepG2 and mESC. MiRNAs revealed novel mechanisms that could contribute in the understanding of the toxicity caused by CDDP exposure. These results are part of an ongoing research project and with the results obtained so far we have partially characterized toxicogenomic responses in different in vitro systems, which contribute in understanding the mechanistic properties of CDDP.

PS 1587 USING A TRANSCRIPTIONAL GENE SIGNATURE TO PREDICT NEPHROTOXICITY *IN VITRO* .

D. DeSilver[1], V. Bonato[2], M. Kuhn[2] and M. T. Pletcher[1]. [1]*Compound Safety Prediction, Pfizer, Inc., Groton, CT and* [2]*Biostatistics, Pfizer, Inc., Groton, CT.*

The kidney is a frequent site of drug toxicity and its injury can be induced by a wide spectrum of agents. Several urine biomarkers exist for detecting drug-induced kidney injury including KIM-1 and osteopontin, but there is a lack of *in vitro* approaches for early identification of compounds that may act as nephrotoxicants. Our strategy consisted of evaluating a published computationally-derived, *in vivo* gene signature (Fielden *et al*, 2005) designed to predict late onset drug-induced renal toxicity using early time point transcriptional data. Despite many of the component transcripts of the signature being poorly characterized genes and representing diverse pathways and functionalities, the drug-induced expression changes of the genes directly correlated with the pathological outcome of a structurally diverse set of nephrotoxicants. The predictivity of the signature model was ~76%. Our intent was to translate this approach *in vitro*, using the same 35 gene signature, with rat kidney tubule cells dosed at a concentration inducing 20% cytolethality where possible. Cells were dosed for 24 hours with a training set of 56 compounds. Fold change data was log transformed, subjected to standard quality control metrics, and run through several predictive models using 200 different training/test splits. Using a Support Vector Machines model with Recursive Feature Elimination, we calculate the sensitivity of the signature to be 70.0% and the specificity to be 71.3%. Despite the *in vivo* derivation of the signature and the relatively small data set, our *in vitro* application is strikingly similar to the *in vivo* performance. In addition, we show that the signature is tissue specific in its prediction with a set of non-nephrotoxicant compounds that induce liver damage *in vivo*. This work establishes a tool for the early detection of a class of high-risk compounds so that they may be removed from development prior to ever reaching a clinical population.

PS 1588 TOXICOGENOMIC COMPARISON OF THE TOXIC EFFECTS OF DIETARY TCDD IN RAINBOW TROUT AND ZEBRAFISH.

Q. Liu[1, 2], M. L. Rise[3], C. A. Struble[4], J. M. Spitsbergen[5], G. Goetz[1], R. J. Hutz[2] and M. J. Carvan[6, 1]. [1]*Great Lakes WATER Institute, Milwaukee, WI,* [2]*Biological Sciences, University of Wisconsin Milwaukee, Milwaukee, WI,* [3]*Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF, Canada,* [4]*Department of Math, Statistics, and Computer Science, Marquette University, Milwaukee, WI,* [5]*Microbiology, Oregon State University, Corvallis, OR and* [6]*School of Freshwater Sciences, University of Wisconsin Milwaukee, Milwaukee, WI.*

The purpose of this project is to use functional genomic and bioinformatic methods to identify molecular biomarkers conserved across divergent teleost species as indicators of the impact of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) exposure. We are using zebrafish and rainbow trout as models to investigate the effects of chronic dietary TCDD exposure on global gene expression anchored to histopathological analysis. Juvenile rainbow trout and young zebrafish were fed dietary TCDD at 0, 0.1, 1, 10 and 100ppb for six weeks. No significant mortality was observed in zebrafish during the six weeks of exposure. However, trout is much more sensitive to TCDD exposure than zebrafish with an LD50 of 10ppb at the end of six weeks and 100% lethality for the 100 ppb diet by 40 days. TCDD caused multiple pathologies in both zebrafish and trout and the severity was both time- and dose-dependent. Histological analysis revealed that by week six significant lesions were found in the liver, kidney, gills, skin, and heart, and caused a delay in the development of the skull and brain. Gene expression analysis was performed using the cGRASP 16K cDNA microarray for trout and NimbleGen 12X135K arrays for zebrafish. A number of genes were dysregulated in trout and zebrafish by dietary TCDD that involve a number of different molecular pathways and some of these may be useful as biomarkers for TCDD effects in the aquatic environment to assess the potential impacts of low-level environmental TCDD in the food chain and on the health of humans and animals. Gene expression network analysis of microarray data from the two species will provide conserved molecular pathways impacted by chronic dietary TCDD exposure for improving our understanding of the mechanism of TCDD toxicity.

PS 1589 IDENTIFICATION OF MECHANISTIC BIOMARKERS OF CARDIAC AND SKELETAL MUSCLE TOXICITY TO AID THE DEVELOPMENT OF TISSUE-SPECIFIC *IN VITRO* MODELS.

W. Dott[1], P. Mistry[2], J. Wright[2] and K. Herbert[1]. [1]*Cardiovascular Sciences, University of Leicester, Leicester, United Kingdom and* [2]*Syngenta, Bracknell, United Kingdom.* Sponsor: R. Peffer.

The primary objective of this work was to discover early markers of striated muscle toxicity, and to aid the development and validation of in vitro model systems to understand and identify muscle toxicants early in the R&D process. In this study, toxicogenomics was used to identify mechanistically-relevant genomic biomarkers of xenobiotic-induced striated muscle toxicity in tissues taken from in vivo studies. Rats were dosed daily via the diet with sulfonyl isoxazoline chemistries for 4 or 28 days. Histological assessments revealed a dose-dependent relationship with myositis and myodegenerative pathology in striated muscle. Transcription profiling of cardiac and skeletal muscle tissue samples taken from sub-toxic dose levels was carried out using Illumina microarray and bioinformatics tools (ArrayTrackTM) to filter and infer biological meaning from the data. After 4 days, subtle but significant ($p<0.05$) gene expression changes were detected in cardiac (n=895) and skeletal muscle (n=718); while at 28 days more significant changes in cardiac (n=2078) compared to skeletal muscle (n=1070) were observed. From these gene lists, a panel of 5 up- and 5 down-regulated mechanistically relevant genomic biomarkers was selected from the most significant differentially expressed genes in cardiac and skeletal muscle. These genes were associated with mitochondrial dysfunction, altered energy metabolism, apoptosis, electrophysiological dysfunction and inflammatory responses, and were explored and validated by qRT-PCR. This work provides the foundation to explore these biomarkers in tissue-specific in vitro model systems to aid prediction of in vivo outcome.

PS 1590 RETROSPECTIVE GENE EXPRESSION ANALYSIS USING FORMALIN-FIXED TISSUE BLOCKS FROM TOXICOLOGICAL STUDIES.

F. v. Landenberg, P. Hewitt, S. O. Mueller and C. S. Schmitt. *Merck Serono, Darmstadt, Germany.*

Toxicogenomic evaluations are becoming more accepted in routine toxicological studies, and typically fresh frozen specimens are employed for microarray analysis. However, large numbers of formalin-fixed, paraffin-embedded (FFPE) tissues have been accumulated in toxicology archives. Thus, it would be beneficial to use these FFPE blocks for molecular toxicology. Although RNA extracted from FFPE tissue is degraded, novel technologies allow their use for gene expression analysis. In this project FFPE tissues of a BMBF-funded project (grant 0313137) were used. Rats were treated with 3 genotoxic carcinogens (N Nitrosodiethylamine, Aflatoxin B1 (AFB), Cupferron), 3 non-genotoxic carcinogens (Carbon tetrachloride, Diethylhexyl Phthalate (DEHP), Clofibrate (CF)) or 3 hepatotoxic non-carcinogens (Diallyl Phthalate, Benzaldehyde, Ketoconazole) for 28 days. RNA was extracted from the paraffin blocks and gene expression was performed using the DASL™ (cDNA-mediated annealing, selection, extension and ligation) assay from Illumina Inc. based on 512 liver toxicity genes. Gene expression was further evaluated using the branch DNA technology with the QuantiGene® FFPE kit. Genes involved in DNA damage and apoptosis, e.g. Cyclin G1 and Cdkn1a, were up-regulated in animals treated with the genotoxic compounds. The Epoxide hydrolase and Aflatoxin reductase, typical for the AFB metabolism, were found to be deregulated in AFB treated animals. Furthermore, animals treated with the peroxisome proliferators, DEHP and CF, highly induced peroxisome proliferating genes and genes involved in lipid and fatty acid metabolism. In addition, genes analyzed with the

bDNA assay corroborated the DASL data. This work showed that the use of degraded RNA from FFPE blocks for microarray studies is possible. Therefore, retrospective analysis without the need for new animal studies could be enabled by using FFPE samples. Furthermore, in studies with unexpected organ toxicities were no fresh frozen tissue is available, toxicogenomic evaluations are still possible and can support mechanistic interpretations.

PS 1591 TRANSCRIPTIONAL DOSE RESPONSE ANALYSIS IN FEMALE MOUSE AND RAT LUNGS FOLLOWING REPEATED INHALATION EXPOSURE TO 2-CHLORO-1, 3-BUTADIENE.

M. W. Himmelstein[1] and R. S. Thomas[2]. [1]*DuPont Haskell Global Centers, Newark, DE and* [2]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Beta-chloroprene (2-chloro-1,3-butadiene), a monomer used in the production of neoprene elastomers, is of regulatory interest due to the production of mouse lung tumors in a two-year rodent bioassay. A significant increase in female mouse lung tumors was observed at 12.8 ppm while a small, but statistically insignificant increase was observed in female rats at 80 ppm. Gene expression microarray and histopathological analysis were performed in the lungs of female mice following exposure at 0.3, 3, 13, and 90 ppm and in female rats following exposure at 5, 30, 90, and 200 ppm. The exposures were selected to span the rodent bioassay concentrations and provide approximately equal target tissue doses in the lung based on total amount metabolized per gram tissue. Exposures were performed for a period of 5 and 15 days. For the mouse, minimal hyperplasia was observed in the terminal bronchioles after 5- and 15 days of exposure at 90 ppm. No significant histopathological changes were observed at any exposure concentration in the female rat lung. Based on traditional analysis of variance, the number of genes with significantly altered expression in the mouse lung at the 5- and 15-day time points was similar and increased with dose. In the rat lung, the number of significantly altered genes was higher at the 5-day time point. Benchmark dose (BMD) methods were used to model the transcriptional dose-response data and calculate dose estimates at which different signaling pathways were altered. The BMD values for the selected signaling pathways were filtered to identify those with a transcriptional dose-response consistent with the tumor response in the rodent bioassay. A small subset of signaling pathways were identified and included changes in glutathione metabolism, oxidative stress, mismatch DNA repair, and amino acid metabolism. The results serve to identify potential modes-of-action for chloroprene for follow-up study.

PS 1592 GENE EXPRESSION PROFILING OF LXR-MEDIATED HEPATIC STEATOSIS IN RATS.

J. M. Maher, W. R. Buck, M. J. Liguori, J. Lai-Zhang, T. Sharapova, S. J. Morgan, E. A. Blomme and Y. Yang. *Investigative Toxicology and Pathology, Abbott Laboratories, Abbott Park, IL.*

The Liver-X Receptor (LXR) is a nuclear receptor that is activated by oxysterols, and LXR mediates key steps in bile acid synthesis. T0901317 is a potent LXR agonist known to markedly increase liver weights, induce fatty acid synthesis, and cause significant hepatic steatosis. The purpose of the current study was to evaluate gene expression changes caused by T0901317 to identify unique genes regulated by LXR agonists that differentiate this profile from other steatotic compounds or from changes observed in starvation-induced steatosis. In the current study, Sprague-Dawley rats were dosed with T0901317 for five days at dosages of 5, 25, and 50 mg/kg/day in PEG400:Tween 80 (80:20 w/w). Livers were collected for both pathology and toxicogenomics, and serum was collected for clinical chemistry. Liver triglycerides (TGs) were markedly increased in a dose-dependent manner, yet serum TGs were significantly decreased after 5 days in an inverse manner, a finding which is seemingly species or timepoint specific when compared to mice. T0901317 caused marked induction of pathways related to sterol regulatory element-binding protein 1-C (SREBP-C) signaling, and these gene changes are generally related to increases in fatty acid and cholesterol biosynthesis. LXR-induced steatosis clustered away from other steatotic models due to marked induction of a few key genes involved in beta-oxidation, including CD36, malic enzyme, SREBP-C, and long chain fatty acyl-CoA synthetase. Furthermore, Angiopoietin-like 3, a key gene in determining serum TG levels in rodents, was not upregulated in response to T0901317 treatment, coinciding with the observed findings. Through gene profiling, recognition of LXR agonism by microarray allows for an early signal that may aid in the prediction of steatosis, which can then trigger the further characterization of experimental compounds that may have LXR-mediated effects on the liver.

PS 1593 CHARACTERIZATION OF THE HEPATIC TRANSCRIPTOME RESPONSE TO A MIXTURE OF LOW MOLECULAR WEIGHT POLYBROMINATED DIPHENYL ETHERS: DISEASE, SIGNATURE, NETWORK, AND PATHWAY ANALYSIS.

S. S. Auerbach[1], K. R. Shockley[1], R. Thomas[2], N. J. Machesky[4], M. K. Vallant[1], C. D. Hebert[3], H. C. Cunny[1] and J. K. Dunnick[1]. [1]*National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC,* [2]*Environmental Health Sciences, University of California, Berkeley, CA,* [3]*Southern Research Institute, Birmingham, AL and* [4]*Battelle Columbus Operations, Columbus, OH.*

Polybrominated diphenyl ethers (PBDEs) are widespread persistent organic pollutants that produce toxicity in the liver, thyroid and brain. As a part of the ongoing NTP studies of the PBDEs, liver transcriptome analysis was performed on liver samples from 22-day old male rats exposed by gavage to a mixture of low molecular weight PBDEs from gestation day 6 to postnatal day 21. Disease-centric enrichment analysis showed differential expression of genes associated with hepatic cholestasis, fibrosis, hepatitis, and hypercholesterolemia. Pertubations of genomic pathways also predicted that the PBDE mixture will be hepatocarcinogenic by non-genotoxic mechanisms. Genomic signature analysis indicated the PDBE transcriptome response is strongly similar to that of phenobarbital, which has been shown to cause liver and thyroid tumors in rats. Coexpression network analysis suggested that the transcription factors CAR, PXR, FOXA3, NRF2, and SREBPF1, as well as the bile salt transporter, NTCP, and the copper transporter, CTR1, play critical roles in PBDE toxicity. Pathway analysis confirmed the role of these genes and their related pathways in the global transcriptional response to PBDEs and further highlighted the role of FXR and LXR signaling. Overall, these data indicate that PBDEs elicit an integrated transcriptional response via perturbation of nuclear receptor, oxidative stress and sterol/cholesterol signaling pathways. Disruption of these pathways has the potential to induce carcinogenic responses in the liver and other organs.

PS 1594 AGE AND SEX DIFFERENCES IN RENAL GENE EXPRESSION DURING THE RAT LIFE CYCLE.

J. C. Kwekel, V. G. Desai, T. Han, W. S. Branham, C. L. Moland and J. C. Fuscoe. *Systems Toxicology/Center for Functional Genomics, U.S. FDA/National Center for Toxicological Research, Jefferson, AR.*

Age- and sex-related susceptibility to adverse drug reactions is a key concern in understanding drug safety and disease progression. Age is a predisposing condition for susceptibility to adverse effects of some drugs. There is also evidence from the study of hepatic disease and cancer to suggest sexually dimorphic susceptibilities as well. We hypothesize that the underlying suite of genes expressed at various developmental and life-cycle stages will impact susceptibility to adverse drug reactions. Thus, understanding the natural expression patterns of genes throughout the life span of the rat surrogate model species in both sexes will inform our assessments of adverse drug reactions. The kidney plays a central role in the pharmacokinetics and excretion of drugs via key molecular transporters. Untreated, male and female F344 rats were sacrificed at 2, 5, 6, 8, 15, 21, 78, and 104 weeks of age. Kidney tissues were collected for histology and gene expression analysis. Whole-genome rat microarrays (Agilent) were used to query global expression profiles. A 2-way ANOVA ($p < 0.0001$) along with 1.5 fold change in relative expression was used to identify 4,061 unique genes that were differentially expressed by sex or age. Principal component analyses (PCA) revealed notable expression profile differences between different age groups with greatest differences observed between 2 and 6 weeks of age. Adult and aging animal expression profiles clustered in a large group with 104 week animals showing slight divergence. PCA loadings analysis prioritized genes having the greatest influence in sex and age related differences in expression and included metabolism and transport genes Cyp1a1, Ugt2b3, Abcg5, Abcb11 and Abca8, among others. K-means cluster analysis identified groups of genes exhibiting similar lifecycle expression profiles. These results suggest specific groups of kidney genes that may underlie specific periods of susceptibility to adverse drug reactions.

PS 1595 GENE EXPRESSION PROFILE OF RAT PRIMARY HEPATOCYTES EXPOSED TO DI(2-ETHYLHEXYL) PHTHALATE OR DIISONONYL PHTHALATE, THE PATH TO GENERATE TOXIGENOMICS DATA AS SUPPORT FOR STRUCTURE ACTIVITY RELATIONSHIPS ASSESSMENTS.

J. M. Naciff, G. J. Overmann, R. Adams, G. J. Carr, J. P. Tiesman and G. P. Daston. *Procter & Gamble, Cincinnati, OH.*

We have evaluated the transcriptional profile of rat primary hepatocytes elicited by Di(2-ethylhexyl) phthalate (DEHP) or Diisononyl phthalate (DINP) exposure, at two doses (250, and 1000 μM) and two time points (24 and 48 h), using the Rat Expression Array 230 2.0 (Affymetrix). This study is part of a project which ultimate goal is to determine whether gene expression profiles from cells in culture (liver-derived) can provide us with sufficient information to support the Structure Activity Relationships (SAR)-based assumptions about biological activity. The genomic response of the rat primary hepatocytes to either DEHP or DINP exposure is both dose- and time-dependent. Trend analysis indicated that the expression of more than 2770 and 1680 probe sets is modified ($p \leq 0.0001$) by DEHP and DINP, respectively. Comparing the expression profile induced by DEHP versus the one induced by DINP, at equivalent exposure times, we clearly identified a relatively large number of genes (represented by 1164 unique probe sets) whose expression changes in the same direction by exposure to these two phthalates. The products encoded by these genes have been associated a great variety of biological processes and molecular functions, particularly enriched in lipid, fatty acid, glutathione and xenobiotic metabolic processes. Further, phthalate exposure results in changes in the expression of unique genes whose products are involved with cell cycle regulation, regulation of programmed cell death (apoptosis), and with stress response. The robustness of the response of the rat primary hepatocytes to these two phthalates indicates that the transcription profiling offers relevant biological data to support the SAR-based assumptions about biological activity, in this in vitro system.

PS 1596 PERCELLOME TOXICOGENOMICS PROJECT AND ITS APPLICATION TO STUDIES ON ANTICANCER AGENTS.

J. Kanno, K. Aisaki, K. Igarashi and S. Kitajima. *Division of Cellular and Molecular Toxicology, National Institute of Health Sciences, Tokyo, Japan.* Sponsor: N. Ryoichi.

Percellome Toxicogenomics Project has been launched to develop a comprehensive gene cascade database/informatics for the mechanism-based predictive toxicology. For this purpose, a normalization method designated as "Percellome" is developed (BMC Genomics 7:64, 2006) to generate mRNA expression values in "copy numbers per one cell" from microarrays and Q-PCR. Thus, data are directly compared among studies and even different organs. Up to now, the time- and dose-dependent alteration of gene expression induced by a single oral exposure in mouse liver and other organs (4 time points x 4 dose levels, triplicate, 48 per chemical) are studied on more than 100 chemicals. Transcriptomic data is expressed as 3-D graphs (time x dose x copies per cell); 45,000 surfaces corresponding to the probe sets of the Affymetrix Mouse Genome 430 2.0 Array. Here, we applied this comprehensive approach for the mechanisms/toxicity of some anticancer agents and related compounds. As a result, thalidomide and 5-FU share the same target(s) in liver and lung involving cdkn1a (p21). However, the time course of the induction of the key mRNA was different (2hr versus 24hr). Accordingly, the molecular networks were different; thalidomide seems to trigger direct signaling towards p21 (p53 network), whereas 5-FU seems to induce signaling towards p21 secondary to pyrimidine metabolism and protein synthesis failure. In addition, both thalidomide and 5-FU induce similar stress responses not only in liver and lung but also kidney and heart indicating possible toxicity to those organs as well. (Supported by Health Sciences Research Grants from the Ministry of Health, Labour and Welfare, Japan)

PS 1597 COMPARISON OF BASAL AND CRVI-MEDIATED SOLUTE CARRIER GENE EXPRESSION IN RODENT DUODENAL EPITHELIUM.

S. Kim[1], C. M. Thompson[2], A. K. Kopec[1], M. A. Harris[2] and T. R. Zacharewski[1]. [1]*Biochemistry & Molecular Biology and Center for Integrative Toxicology, East Lansing, MI and* [2]*ToxStrategies, Inc., Katy, TX.*

Hexavalent chromium (CrVI) is structurally similar to phosphate and sulfate ions and readily enters cells via anion transporters. Chronic administration of high doses of sodium dichromate dihydrate (SDD) leads to intestinal cancer in mice, but not in rats. To further evaluate species-specific differences in CrVI carcinogenicity, duodenal epithelia of female B6C3F1 mice and Fisher rats were examined after 7 days of continuous exposure to 0.3-520 mg/L SDD in drinking water. Whole genome 4x44K Agilent two-color oligonucleotide arrays identified basal expression of 289 unique solute carrier (SLC) transporter orthologs in control animals. SLC intensity values were normalized to the average signal intensity for all SLC orthologs. Overall, SLC expression levels were comparable between species with some notable exceptions. For example, there were species-specific differences (~86-20-fold) in the basal expression of SLC *35c1, 7a7, 6a20, 25a25, 5a11, 2a2, 25a4, 35d1, 25a25*, and *35e2* in the mouse, while basal levels of SLC *25a4, 9a1, 30a3, 15a1, 44a4, 26a3, 4a4, 24a6, 35a4,* and *39a8* were ~82-15-fold higher in the rat. Exposure to SDD differentially regulated 119 mouse and 48 rat SLC orthologs (|fold change|>1.5, P1(t)>0.999), of which 35 were commonly regulated in both species. The uptake of CrVI is believed to be mediated by Slc4a family, in agreement with high basal expression of *Slc4a2* (3.5-fold) and *Slc4a5* (2.1-fold) in the mouse, and *Slc4a4* (13.4-fold) and *Slc4a7* (21.3-fold) in the rat. SDD treatment did not affect expression of SLCs 4a2/5 in the mouse, but significantly repressed SLCs 4a4/7 in the rat at the highest doses. *SLC4a1*, the putative CrVI transporter, exhibited low basal expression and was not differentially regulated by SSD. The results suggest that CrVI exposure may alter SLC transporter expression. Differences in basal SLC expression and regulation could partially explain the observed carcinogenic phenotype differences between rats and mice.

PS 1598 NOVEL SHORT TERM PREDICTION SYSTEM FOR CARCINOGENICITY OF CHEMICALS BY HEPATIC TRANSCRIPT PROFILING IN A 28-DAY REPEAT-DOSE TOXICITY STUDY.

F. Saito[1], H. Matsumoto[1], M. Takeyoshi[1], Y. Yakabe[1] and T. Shirai[2]. [1]*Chemicals Assessment and Research Center, Chemicals Evaluation and Research Institute (CERI), Japan, Saitama, Japan and* [2]*Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.*

Carcinogenicity is one of the most important endpoint of chemical safety for not only pharmaceutical compound but industrial chemicals. However, rodent tests for carcinogenicity of chemicals such as a traditional 2-year study, requires large number of animals and large amount of compounds. In this study, we have been trying to develop a highly accurate and wide applicable prediction method of chemical carcinogenicity with short term toxicity test up to 28 days. We analyzed the gene expression profiles in livers of Fischer 344 rats administered with 47 carcinogenic and 26 non-carcinogenic compounds as test data set. Gene expression of the livers were analysed using a custom oligo microarray, NEDO-ToxArray III and Affymetrix GeneChip®. Functions and networks analyses of up- or down-regulated genes were conducted using the Ingenuity Pathways Analysis (IPA) software. A prediction formula was developed by most plausible method using gene expression profiles of test data set with SVM approach. The established formula showed a concordance of 94.4% with test data set, and the formula was validated by application of 18 compounds as the training data with a concordance of 83.3%. Furthermore, gene expression data obtained from Sprague-Dawley rat treated with short-term toxicity test with known carcinogens and non-carcinogens were applied to the prediction formula, and all hepatic carcinogens and non-carcinogens were accurately predicted. In addition, key genes used in the prediction formula showed a common gene network contained tumor suppressor gene, p53 concerned with cell cycle, DNA repair, and apoptosis. Thus, we developed a promising short term prediction system for carcinogenicity of chemicals using gene expression profiles in livers of rats before neoplastic changes.

PS 1599 TIME AND CONCENTRATION DEPENDENT REGULATION OF THE TRANSCRIPTOME AND THE PHENOTYPE OF A MOUSE LIVER CELL LINE EXPOSED TO BAP.

D. Jannuzzi Madureira[1, 2], J. Michaelson[3], A. Beyer[3], S. Trump[4], I. Lehmann[4] and K. Schirmer[1, 2]. [1]*Eawag, Swiss Federal Institute of Aquatic Science and Technology, Duebendorf, Switzerland,* [2]*Swiss Federal Institute of Technology Zürich, Switzerland, Zuerich, Switzerland,* [3]*Biotechnology Center TU Dresden, Dresden, Germany and* [4]*Helmholtz Centre for Environmental Research, Leipzig, Germany.* Sponsor: M. R. Embry.

The aim of the systems biology initiative, "From contaminant molecules to cellular response: system quantification and predictive model development", is to build a model of interactions between cells and the toxic chemical Benzo-a-Pyrene (BaP). As part of this initiative, this project aims to identify the regulation of genes by BaP in a time and concentration-dependent manner and relate the regulation to cellular distribution of BaP and the physiologic or toxicologic cellular response. The well

characterized mouse hepatoma cell line, Hepa1c1c7, was chosen as a cellular model. In a first step, two concentrations (50 nM and 5 μM) and four time points (2, 4, 12 and 24 h) were used for comprehensive transcriptome analysis. Thus, RNA was isolated and applied to Affymetrix exon arrays. Analysis showed a total of 2000 genes to be significantly regulated either by concentration or by time and concentration. About 89 regulated genes are transcription factors, raising the possibility of transcription factor cross-talk in BaP signalling. Genes related to transcription/translation, cell adhesion and DNA repair are among the regulated genes. Some of the robustly regulated genes are those encoding for cytochrome P450 enzymes and the TCDD-inducible poly(ADP-ribose) polymerase, Tiparp. These are known targets of the aryl hydrocarbon receptor (AhR), which is activated by BaP. To start to link these regulations in the transcriptome to the cellular phenotype, changes in cell number were explored over 48 hours of exposure to up to 5 μM BaP. Indeed, cell number increased over time with no apparent differences up to 500 nM BaP. At 5 μM BaP, however, cell number stayed constant over the entire exposure time, thereby indicating disruption of the cell cycle. Our next goal is to identify how cellular functions change if Tiparp is knocked down.

PS 1600 TRANSCRIPTOMICS ANALYSIS OF NANOPARTICLE-CONTAINING HAIR DYES.

D. A. Sarigiannis, G. Cimino Reale, E. Marafante, B. Casati, R. Brustio and A. Collotta. *Institute for Health and Consumer Protection, European Commission - Joint Research Centre, Ispra, Varese, Italy.* Sponsor: L. Birnbaum.

The overall objective of this study was to explore the applicability of transcriptomics for risk assessment of mixtures of chemicals in consumer products such as cosmetics and in particular hair dyes so as to develop a systems toxicology framework for consumer product risk assessment. In particular, this approach is expected to identify genetic biomarkers of exposure and early effects related to chronic exposure to low doses of commercially relevant hair dye preparations. According to the study design these biomarkers would be identified through the use of whole genome gene expression micro-arrays and corroborated with quantitative PCR using micro-fluidic cards for specific gene families and biological processes.
The study comprised in vivo analysis on urinary bladder and on skin from rat for the identification of gene expression patterns in target organs, and quantitative PCR using micro-fluidic cards for specific inflammation genes.
Comparison was made with formulations including reactants usually applied in the products application (e.g. p-phenylendiamine and atninophenol). Three different formulations were tested: the active substance (p-phenylendiamine, PPD); a commercial hair dye formulation using PPD as its principal active ingredient (HD); a similar commercial hair dye using nanoparticle-bound PPD for better dyeing and duration effects (HD nano).
The chemicals were applied both on the skin and on the hair of the treated rats. A number of biological processes that were significantly induced compared to the controls after treatment are characteristic of specific products. Blood circulation, clotting and vesicular transport were induced by HD nano only, suggesting that the overall chemical mixture in the HD nano preparation behaves differently than the conventional HD preparation. Blood circulation features should be tested when assessing the toxic potency of HD nano. Moreover, several metabolic processes were induced after treatment with HD nano, including fatty acid, cholesterol, amino acid and nitrogen metabolism.

PS 1601 A TOXICOGENOMIC COMPARISON OF PRIMARY *VERSUS* PHOTOCHEMICALLY ALTERED AIR POLLUTANTS IN HUMAN LUNG CELLS.

J. E. Rager[1], K. G. Sexton[1], L. Smeester[1], I. Jaspers[2] and R. C. Fry[1]. [1]*Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC and* [2]*Center for Environmental Medicine, Asthma, and Lung Biology, School of Medicine, University of North Carolina, Chapel Hill, NC.*

Outdoor air pollution contributes significantly to global increases in mortality, particularly within urban environments. Studies have investigated lung responses caused by single pollutants, such as individual gases or particulate matter. There is, however, limited knowledge on the mechanisms underlying health effects resulting from exposure to pollutant mixtures. Toxicogenomic analyses can increase our mechanistic understanding through the study of gene expression alterations and associated molecular pathways. Here, we set out to compare and contrast responses of lung cells exposed to primary pollutants relative to photochemically altered (PCA) pollutant mixtures found in urban atmospheres. We hypothesized that lung cells exposed to PCA pollutants would show increased modulation of inflammatory-associated genes and pathways relative to primary air pollutants. Human lung epithelial cells were exposed to either primary or PCA air mixtures. mRNA expression changes were assessed using microarrays and confirmed using RT-PCR on a subset of genes. There was a massive difference in cell response with primary air pollutants altering the expression levels of 19 genes, while PCA pollutants altered 709 genes. Functional and molecular analyses of the altered genes showed that pollutant exposure modifies the signaling of pathways associated with cancer and inflammation. To confirm the altered inflammatory response, protein was collected and interleukin-8 was measured at significantly increased levels. Our study shows for the first time that photochemically produced secondary pollutants initiate significantly more robust changes in gene expression patterns in comparison to primary components of air pollution. This difference may, in part, explain the health effects related to air pollutant exposure.

PS 1602 UNCOVERING TRANSCRIPTIONAL REGULATORS OF KIDNEY INJURY MOLECULE-1 IN CISPLATIN-INDUCED KIDNEY TOXICITY.

M. P. Wagoner, J. Milano, D. Brott, D. Thurman, Y. Dragan, L. B. Kinter and B. Jeffy. *Safety Assessment U.S., AstraZeneca, Wilmington, DE.*

Kidney Injury Molecule 1 (KIM-1) is emerging as an important biomarker for drug induced histologically defined renal tubular injury (DIKI), as it is a more sensitive and more accurate measure of DIKI than current clinically available tests. Nephrotoxic compounds such as cisplatin or gentamicin increase both tubular KIM-1 mRNA and urinary KIM-1 protein. While it is understood that urinary KIM-1 protein levels are driven by increased KIM-1 transcription, the transcription factors responsible are as of yet unknown. We used an acute dose model of cisplatin induced nephrotoxicity to identify putative KIM-1-inducing transcription factors, examining kidney transcriptional profiles from 54 rats from 3 different strains following varying doses of cisplatin. Genes induced by cisplatin in a manner resembling KIM-1 expression patterns were identified implying that these genes may have common transcriptional regulators (Pearson correlation $p<10\text{-}10$). Using whole genome rVista analysis, we probed the promoters of those genes that showed KIM-1-like expression patterns for transcription factor binding sites that were statistically over-represented and identified the transcription factor hepatocyte nuclear factor fork head homolog 3 (HFH3, also known as FOXI1) binding sites as significantly enriched($p<10\text{-}32$). HFH3 is expressed almost exclusively in the kidney, and plays critical roles in terminal nephron differentiation. The gene encoding KIM-1 mRNA, HAVCR1, also contains evolutionarily conserved consensus HFH3 binding sites. To determine if HFH3 function is indeed altered in cisplatin-induced DIKI we evaluated the expression of 160 predicted HFH3 target genes before and after cisplatin treatment. Gene set enrichment analysis of these genes finds that like KIM-1, many HFH3 gene targets showed significant changes in expression following cisplatin treatment, implying that HFH3 function is altered in response to DIKI. Together, these data suggest a possible role for HFH3 in driving KIM-1 expression in response to cisplatin-induced kidney injury.

PS 1603 CORRELATION BETWEEN TOXICOGENOMIC PROFILING AND TRADITIONAL SAFETY BIOMARKERS.

T. Wang[1], M. Papoutsi[2], M. Decristofaro[3], M. Keselica[3], E. Skuba[3], R. Spaet[3], P. Moulin[2], F. Pognan[2], A. Wolf[2] and W. Kluwe[1]. [1]*Translational Sciences, Novartis Institute of Biomedical Research-USA, Emeryville, CA,* [2]*Translational Sciences, Novartis Institute of Biomedical Research-Switzerland, Basel, Switzerland and* [3]*Novartis Institute of Biomedical Research-USA, Easter Hanover, NJ.*

Integrated interpretation of the hepatic safety biomarkers ALT, AST and liver histopathology have been used to identify drug-induced hepatotoxicity in preclinical animal models. Poor correlations between these safety markers have been reported. Elevated serum ALT and AST levels without concurrent histopathological evidence of liver necrosis especially confound preclinical interpretation of these data. BLZ945 is being developed as a palliative care pharmaceutical designed to inhibit tumor-induced bone osteolysis in cancer patients with bone metastasis. In the preclinical safety evaluation studies, oral administration of BLZ945 to rats and monkeys caused moderate, dose-dependent elevations in serum ALT and AST, indicating potential liver damage. However, liver hypertrophy was the only pathological finding in rats, and there was no evidence of any morphological changes in the livers of the monkeys. A subsequent kinetic study demonstrated that daily dosing of BLZ945 did not elevate ALT immediately. Instead, it took a week for the ALT levels to increase approximately 3-fold. Continued treatment with BLZ945 for 6 weeks caused elevation of ALT up to 6-fold, yet hepatocyte necrosis was not detected by histopathological examination of the liver. Toxicogenomic profiling of the livers of rats and monkeys treated with BLZ945 demonstrated that the genes associated with early response to liver injury, apoptosis/necrosis, inflammation, oxidative stress, and metabolic enzymes were upregulated. Our results suggest that under conditions where there is a poor correlation between serum ALT/AST levels and morphological changes in the liver, toxicogenomic profiling might be a useful tool

to confirm a hepatotoxic property of the compound in question, which might otherwise be disregarded based on traditional safety biomarker and histopathology screening.

PS 1604 MUSCLE IMPLANTED PELLETS OF TUNGSTEN/NICKEL/COBALT, NICKEL, OR TANTALUM: A 12 MONTH STUDY OF METAL MOBILIZATION AND MICROARRAY EXPRESSION.

D. I. Bannon[1], A. B. Rosencrance[2], W. E. Dennis[2], W. Bao[3], C. Tsu-Ming[3], R. D. Wolfinger[3] and E. J. Perkins[4]. *[1]Directorate of Toxicology, U.S. Army Public Health Command, Aberdeen Proving Ground, Aberdeen, MD, [2]Department of Chemistry, U.S. Army Center for Environmental Health Research, Fort Detrick, MD, [3]SAS Institute, Inc., Cary, NC and [4]Environmental Laboratory, U.S. Army Engineer Research and Development Center, Vicksburg, MS.*

Alloys of metals can include nickel, a known cancer inducing agent. In a 12 month study, a single pellet of either a tungsten/nickel/cobalt alloy (WNiCo), pure nickel (Ni), or tantalum (Ta) control were implanted, under anesthesia, into the leg muscle of C3H mice. At 3 week intervals up to 53 weeks, mice (n=5) were euthanized and samples taken for serum, urine (pooled), or microarray analysis (muscle tissue around pellet). Results show metals were excreted in urine at high rates in the first months of the study, after which elimination dropped significantly. Serum samples also showed metal concentration increases in the first months of the study related to treatment (WNiCo, Ni) with lower concentrations in the later months. Little or no metal was mobilized from animals treated with Ta control pellets. Microarray analysis showed that there were patterns of gene expression related to treatment and/or time. These results indicate that there is significant metal mobilization from WNiCo and Ni pellets and that these metals cause perturbations in gene expression that is a function of the metal type.

PS 1605 EXPOSURE TO HEAVY METALS CAUSES DISTINCT CHANGES IN THE GENE EXPRESSION PATTERN OF A RAT LIVER-DERIVED CELL LINE.

M. Permenter[2], J. A. Lewis[1] and D. A. Jackson[1]. *[1]U.S. Army Center for Environmental Health Research, Fort Detrick, MD and [2]Excet, Inc., Fort Detrick, MD.*

Nickel (Ni), cadmium (Cd), cobalt (Co), and chromium (Cr) are toxic industrial chemicals with an exposure risk in both occupational and environmental settings. While these substances are known to have adverse health effects, the detailed mechanisms of toxicity remain unclear. DNA microarrays measure the abundance of thousands of gene transcripts simultaneously, providing a snapshot of what is occurring inside a cell at a given moment. To elucidate the mechanisms of toxicity of Ni, Cd, Co, and Cr, H4-II-E-C3 rat liver-derived cell lines were treated with several concentrations of each metal based on initial viability assays and gene expression patterns were determined with DNA microarrays. Probe sets with an FDR of ≤ 0.001 and changing by at least 1.8 fold in one treatment group compared to the control were considered differentially expressed.
The modulated genes were involved in biological processes and pathways including oxidative stress response, apoptosis, cell cycle regulation, and hypoxic response. Comparative analysis identified both common and unique modulated transcripts and perturbed pathways. All the metals affected genes involved in oxidative stress, while chromium most strongly affected those involved in DNA damage and repair, nickel triggered a hypoxic response, and cadmium activated the unfolded protein response. Therefore, we identified novel targets within known toxicity mechanisms that have potential as possible intervention points for Ni, Cd, Co, and Cr toxicity.
Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. The research described herein was sponsored by the U.S. Army Medical Research and Materiel Command, Military Operational Medicine Research Program.

PS 1606 TRANSCRIPTIONAL SIGNATURES FOR CHEMICALLY INDUCED HEPATOXICITY: *IN VIVO* PREDICTIVE POWER OF 3D LIVER CO-CULTURE OVER 2D HEPATOTOXICITY.

B. A. Naughton[1], R. S. Thomas[3], L. New[1], C. I. Pearson[1], A. H. Roter[2] and D. R. Applegate[1]. *[1]RegeneMed Inc., San Diego, CA, [2]Entelos, Inc., Foster City, CA and [3]Genomics, The Hamer Institutes for Health Sciences, Research Triangle Park, NC.*

The two year rodent bioassay is often used to assess chronic toxicity of compounds. Because of its high cost and lengthy time, many chemicals of concern have not been tested. To increase throughput primary hepatocyte cultures are often used but they have serious limitations including partial expression of xenobiotic metabolizing enzymes and short-term function. A three-dimensional (3D) organotypic liver co-culture (3DLC) provides a physiologically relevant model of intact liver by culturing all hepatic cells on a 3D scaffold. These co-cultures form a tissue that maintains liver functions including cytochrome P450 induction for months. Chemically induced transcriptional profiles have been derived from 2D hepatocytes and 3DLC from the same donor along with in vivo dosing of sibling rodents to evaluate the ability of the two in vitro models to predict in vivo function. Microarray data was generated from cultures exposed to 12 toxicants representing five different compound classes (receptors AhR, CAR/PXR, PPAR; inflammation and hypoxia). The 2D hepatocyte cultures lack the multicellular milieu and structural organization of 3DLC and thereby are not successful in predicting complex toxicity signatures. Differential regulation of pathways known to be regulated by these compounds was similar between 3D cultures and in vivo liver: PPARα agonists upregulated genes in the fatty acid beta oxidation pathway; CAR/PXR agonists upregulated Cyp3A and Cyp2B; and AhR agonists upregulated Cyp1A1 and other AhR-responsive genes. Inflammatory agents induced an acute phase response in 3D cultures similar to that induced in vivo; not possible with 2D hepatocytes that lack inflammatory liver cells. Comparison to DrugMatrix (Entelos, Inc) rodent transcriptional database, showed that the 3DLC treatments scored positive against their respective in vivo derived classifiers whereas 2D prediction was limited.

PS 1607 COMPARISON OF BDE-47 AND ITS HYDROXYLATED ANALOGUES ON THE DEVELOPMENT OF EMBRYONIC ZEBRAFISH.

C. Y. Usenko[1, 2] and E. D. Bruce[1, 3]. *[1]Biology, Baylor University, Waco, TX, [2]Biomedical Studies, Baylor University, Waco, TX and [3]Department of Environmental Science, Baylor University, Waco, TX.*

Polybrominated diphenyl ethers (PBDEs) are a class of compounds that have been used as flame retardants for the last 30 years. Recently, there has been growing concern over the potential for PBDEs to disrupt development. Furthermore, hydroxylated analogues have been detected in environmental and human samples worldwide. Currently, there is little information regarding the toxicity of these hydroxylated PBDEs, although there is significant concern that they may pose a greater risk from exposure than the parent compound. In this study, BDE 47 and three hydroxylated BDE 47 congeners were evaluated for developmental effects in zebrafish. Embryos were dechorionated at 6 hours post fertilization (hpf) and exposed to a concentration gradient of each of the congeners. BDE 47 elicited remarkably different effects than the hydroxylated BDE 47 compounds (3-OH-BDE 47, 5-OH-BDE 47, 6-OH-BDE 47). Embryos exposed to BDE 47 had alterations in spontaneous movement at 24 hpf, a curved body axis starting around 120 hpf, and resulted in mortality at 168 hpf when exposed to 5 μg/L and higher concentrations. Conversely, hydroxylated BDE 47 exposures did not affect behavior at any concentration. All congeners investigted, however, delayed development in a concentration-dependent manner. At lower concentrations where the embryos did continue to develop at the normal rate, embryos developed pericardial edema and fin malformations. Furthermore, the concentration-responses varied significantly between the three hydroxylated congeners with embryos most sensitive to 6-OH-BDE 47 exposures and least sensitive to 3-OH-BDE 47 exposures. The addition of the hydroxyl group greatly increased the toxic potential of BDE 47. The location of the hydroxyl group on the PBDE structure also influenced its biological activity. The results of this study indicate that there is a specific interaction between hydroxylated PBDEs and biological systems that is significantly different than that which is observed for the parent compound.

PS 1608 STUDY OF ANTI-DIABETICS AND LMA BY MMP CHANGES ON HEPG2 CELLS USING LASER SCANNING CYTOMETER AND HYPERCYT-CYAN FLOW CYTOMETER.

A. Juan-Garcia[1], J. Robinson[1, 2], V. Davisson[3], C. Juan[4], M. Ruiz[4] and G. Font[4]. *[1]Basic Medical Science-PUCL, Purdue University, West Lafayette, IN, [2]Biomedical Engineering-PUCL, Purdue University, West Lafayette, IN, [3]Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN and [4]Medicina Preventiva i Salut Publica, Ciencies de l' Alimentació, Toxicologia I Medicina Legal, Valencia University, Burjassot, Valencia, Spain.*

Antidiabetics, thiazolidinediones (TZD) and biguanides (BD) are involved in the reduction of fast plasma glucose and insulin levels. On the other hand, LMA as fibrates (FB) and statins (ST) are used in the treatment of hyperlypidaemias. Mitochondrial impairment is increasingly implicated in the etiology of toxicity caused by either some antibiabetics or LMA. 5 hypoglucemic compounds, 3 TZD (Rosiglitazone, Troglitazone and Pioglitazone) and 2 BD (Metformin and Phenformin) and 6 lipid modifying agents, 3 ST (Simvastatin, Pravastatin and

Fluvastatin) and 3 FB (Ciprofibrate, Fenofibrate and Gemfibrozil) were studied, using a high-content toxicity screening (HCS) by two different systems for the analysis of assorted drugs in HepG2 cells. Both systems were used as implemented via: Laser Scanning Cytometry (LSC) and with the HyperCyt instrument matched with a CyAn cytometer. HCS data comprised of measurement of mitochondrial membrane potential (MMP, by TMRM), plasma membrane permeability (ToPro-3), and DNA content, nuclear circularity and area (Hoechst 33342). Disruption of MMP is the one of the earliest indicators for the mitochondrial toxicity. An altered mitochondrial response in HepG2 was observed after 24h. A decline of MMP was accompanied by decreasing cell viability. ST and TZD presented a variable MMP response within the class of agents with simvastatin and rosiglitazone having the most influence on MMP. FB and BG responses were consistent. Similar IC50 values were obtained for the eleven compounds by both systems. Statistical analysis tools were developed for ungating and these were compared with traditional gating. Funding: Postdoc Research Mobility Grant (EX2008-0452) USA.

PS 1609 EFFECTS OF GESTATIONAL EXPOSURE TO PFOA ON PPAR PROTEIN AND MRNA EXPRESSION IN VITAL ORGANS OF FETAL AND POSTNATAL MICE.

A. M. Watkins, C. R. Wood, K. P. Das, C. Lau and B. D. Abbott. *U.S. EPA, Research Triangle Park, NC.*

Perfluorooctanoic acid (PFOA) is developmentally toxic, causing in utero and neonatal mortality, and altering development and growth in mice. PFOA activates peroxisome proliferator-activated receptor (PPAR)α and PPARα signaling is required for toxicity. This study examines the expression of PPARα, β, and γ in fetal and postnatal mice. Timed pregnant CD-1 mice were dosed orally from gestational day (GD) 1-17 with vehicle or 5 mg PFOA/kg. Tissues were collected on GD14, GD17, postnatal day (PND) 1, 7, 14, 21, and 28, and divided for preparation of RNA and protein. qPCR and Western blot data were normalized to internal controls (GAPDH, β-actin). PFOA exposure altered PPAR expression in liver, decreasing expression of mRNA and protein for PPARα and β on PND14. Other changes in liver included decreased mRNA for PPARα, β, and γ on PND1, decreased PPARβ mRNA on PND1-21, and increased PPARα mRNA on PND21 and PPARγ mRNA on PND21-28. In the heart, PFOA exposure decreased PPARα mRNA on PND14 and protein on PND21. Heart PPARβ mRNA decreased on PND14 and 28, and PPARγ mRNA increased on PND1. In PFOA-exposed kidney, PPARγ mRNA was increased on PND14 and PPARβ protein decreased on PND28. In the stomach, PFOA exposure decreased PPARγ mRNA and protein on PND14 and mRNA remained depressed on PND21. PFOA increased PPARβ protein levels in stomach on PND21. In the intestine, PFOA increased PPARγ mRNA on PND1 and 14, while PPARβ mRNA was reduced on PND21. In spleen, thymus, and lung, PFOA increased PPARα mRNA on PND1 and PPARβ mRNA was also increased on PND1 in spleen and thymus. PFOA decreased adrenal expression of PPARγ mRNA on PND14. In addition, PPAR-, CAR-, and PXR-regulated genes were examined and effects of PFOA on expression differed between tissues. The profiles of PPAR expression and the responses to PFOA are complex. These results will be valuable for the further elucidation of PFOA's mode of action for developmental toxicity.
[This abstract does not necessarily reflect US EPA policy.]

PS 1610 DEVELOPMENTAL TOXICITY OF LEFLUNOMIDE AND ITS METABOLITE TERIFLUNOMIDE IN ZEBRAFISH EMBRYOS.

A. Mattsson, K. Vikholm, E. Ullerås and A. Oskarsson. *Biomedical Sciences and Veterinary Public Health, The Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Leflunomide is a prodrug used in treatment of rheumatoid arthritis. Leflunomide is rapidly metabolized to the active metabolite teriflunomide, which is long-lived in plasma and induces embryolethality and teratogenicity in rats and rabbits at plasma levels that are within the therapeutic range. We have studied developmental toxicity of leflunomide and teriflunomide in zebrafish embryos. Embryos were exposed to leflunomide (0.1 - 5 μg/mL) and teriflunomide (0.1 - 10 μg/mL) from 2 hours post fertilization (hpf) and observed at 8, 24, 78 and 72 hpf for mortality and developmental parameters. The two compounds induced similar effects, but leflunomide was more potent. A dark-appearing yolk and heart malformations were the most sensitive endpoints for both leflunomide and teriflunomide (LOAEL: 0.1μg/mL and 0.5μg/mL, respectively). Effects at higher concentrations included oedema, low blood circulation, developmental delay, lack of pigmentation, various malformations and lethality. The impact of chorion as a protective barrier was investigated by exposing dechorinated and intact embryos to leflunomide and teriflunomide at 0.1 and 0.5 μg/mL, respectively. No differences in effects were found between intact and dechorinated embryos. Sensitivity at various developmental stages was studied by exposing embryos to leflunomide (2 and 5 μg/mL) for 2 hrs starting at 2, 12, or 24 hpf. Most abnormalities induced were the same as after continuous exposure, which stated at 2 hpf. However, starting exposure at later developmental stages resulted in less pronounced effects. The results show that both leflunomide and its metabolite teriflunomide are potent teratogens in zebrafish, leflunomide being the more potent of the two. The LOAEL of teriflunomide in the present study (0.5 μg/mL) is approximately 100 times lower than steady-state plasma levels in patients (Brent, 2001). Two hours exposure was sufficient to cause teratogenic effects and the chorion did not act as a protective barrier against the compounds.

PS 1611 IMPACT OF DIFFERENT OILY VEHICLES ON TESTICULAR TOXICITY OF DI-BUTYL PHTHALATE (DBP).

A. J. Martino-Andrade[1, 2], A. S. Lourenço[2], B. C. Minatovicz[1], M. F. Kienast[1], R. N. Morais[1] and P. R. Dalsenter[2]. [1]*Physiology, Universidade Federal do Paraná, Curitiba, Brazil and* [2]*Pharmacology, Universidade Federal do Paraná, Curitiba, Brazil.* Sponsor: R. Landsiedel.

In rats, reproductive adverse effects resulting from developmental exposures to DBP and other active phthalates seem to be highly variable depending on rat strain and other laboratory conditions. One possible component responsible for such variability could be the type of oily vehicle used in experimental studies. In addition, there are reports indicating that phthalates could act either as pro-inflammatory or anti-inflammatory compounds, and that maternal dietary supplementation with omega-3 fatty acids, such as those present in canola and fish oil, could alter the toxicity of pthalates. In the present study, we evaluated the impact of different oily vehicles on testicular effects induced by DBP in male rats fetuses. Pregnant rats (n=11-13/group) were treated by oral route (gavage) with DBP diluted in different vehicles from day 13 to 20 of gestation. A total of 6 experimental groups were used - 3 control groups: 5 mL/Kg of oil (corn, canola or fish); and 3 treated groups: DBP 500 mg/Kg/day diluted in corn, canola or fish oil. Dams were sacrificed on day 20 of gestation and the anogenital distance and intratesticular testosterone levels were evaluated in male offspring. All DBP treated groups exhibited a significant reduction in both anogenital distance and intratesticular testosterone levels, when compared to the groups that received the correspondent vehicles. There were no differences between the DBP groups, indicating that the three different vehicles used (corn, canola and fish oil) had no impact on testosterone production and anogenital distance, an external marker of androgen action. In addition, no significant differences were observed among vehicle-only groups (controls). It remains to be determined whether these different vehicles can have any impact on other phthalate-induced alterations such as gonocyte multinucleation in fetal testis. Moreover, it is important to establish the impact of longer periods of maternal supplementation (e.g., prior to pregnancy).

PS 1612 *IN UTERO* EXPOSURE TO BISPHENOL A INDUCES CHANGES IN THE DEVELOPING MAMMARY GLAND.

J. LaRocca, J. Pietruska and M. Hixon. *Brown University, Providence, RI.*

Bisphenol A (BPA), a chemical widely used in the production of plastics, is the subject of recent controversy as to its effects as a reproductive toxicant. We are investigating if *in utero* exposure to low to moderate doses of BPA induces phenotypic and/or transcriptional changes in the adult mammary gland. Pregnant C57/Bl6 mice were exposed to either sesame oil as a negative control, a low dose (50μg/kg) of BPA, a moderate dose (1,000μg/kg) of BPA, or diethylstilbestrol (2.0μg/kg) as a positive control via oral gavage from gestational days 10 to 16. Offspring were sacrificed at either postnatal day (PND) 25 or PND 56. Whole mount analysis indicated that both mammary gland ductal outgrowth and the terminal end bud population were significantly decreased at PND 25 for the 50μg/kg BPA and DES exposure groups, but were unchanged for the 1,000μg/kg BPA group. However, by adulthood mammary glands exposed *in utero* with BPA at both the moderate (50 μg/kg) and high (1,000 μg/kg) dose or DES (2 μg/kg) resulted in increased ductal outgrowth as compared to sesame oil control glands. Real-time RT-PCR revealed statistically significant increased expression of progesterone receptor, estrogen receptor alpha, and g-coupled protein receptor 30 (GPR30) in PND 25 mammary glands exposed *in utero* with 1,000μg/kg BPA. Interestingly, by adulthood GPR30 mRNA levels were significantly decreased in all exposure groups. Initial evidence also suggests that mice exposed to 30 mg/kg Dimethylbenz[α]nthracene at PND 56 that were previously exposed *in utero* to 1,000 μg/kg BPA have increased mammary hyperplasia by one year of age. We are currently conducting in vitro experiments with BPA to differentiate between classical and non-classical estrogenic signaling pathways. Our results suggest that *in utero* exposure to BPA alters postnatal mammary gland development, causing a permanent change in mammary gland structure and possibly increased susceptibility to cancer development following carcinogen exposure. Furthermore, the pathways through which BPA elicits its response appear to be dependent on the dose, resulting in a non-monotomic dose response.

PS 1613 AN INVESTIGATION OF THE CLEARANCE OF ^{14}C-LABELED VITAMIN K IN CONTROL AND MCCP-TREATED FEMALE CD RATS.

R. H. Powrie[1], D. G. Farrar[2] and C. R. Elcombe[1]. [1]*CXR Biosciences Ltd., Dundee, United Kingdom and* [2]*Ineos Chlor Ltd., Runcorn, United Kingdom.*

Previously it has been shown that oral administration of medium chain chlorinated paraffins (MCCPs) to female rats prior to pregnancy and during gestation markedly decreased the maternal plasma and milk concentrations of Vitamin K. This in turn contributed to Vitamin K deficiency in the neonatal pup and increased mortality due to internal haemorrhaging. The study presented was aimed at determining the fate of a single oral or intraperitoneal (i.p.) dose of ^{14}C-Vitamin K (5mg/kg bodyweight) to female CD rats following a schedule of 14 daily oral doses of corn oil (control) or Cereclor S52 (MCCP) at a dose of 1g/kg bodyweight. Plasma analysis from the control and MCCP-treated rats after oral administration of ^{14}C Vitamin K showed a significant decrease in the circulating amounts of radiolabelled material in the MCCP-treated rats. This decrease in plasma levels was not seen after the i.p. administration of ^{14}C-Vitamin K. The recovery of radiolabelled material in the urine, when calculated from the total amount of ^{14}C-Vitamin K dosed orally, was significantly reduced (approximately 50%) in the MCCP-treated rats when compared to the control rats. There was no significant reduction in the levels of radiolabelled material in the urine of the MCCP-treated group after i.p. dosing of ^{14}C-Vitamin K.
48 hours after orally dosing ^{14}C-Vitamin K, 95% of the total radiolabelled material could be accounted for in the faeces from the MCCP-treated rats whereas, in the control group, only 70% could be accounted for. There were marked decreases in the amounts of radiolabelled material in the whole liver, the whole kidney and the amounts per gram of body fat from the MCCP-treated rats when compared to the controls in those receiving ^{14}C-Vitamin K by the oral, but not the i.p. route. These results suggest that prior treatment of female CD rats with MCCPs decreases the intestinal uptake of orally administered ^{14}C Vitamin K. The mechanisms for the decreased uptake of Vitamin K are being investigated.

PS 1614 KETAMINE INDUCES MOTOR NEURON TOXICITY AND ATTENUATION OF HEART RATE IN ZEBRAFISH.

J. Kanungo, E. M. Cuevas, Z. He, S. F. Ali and M. G. Paule. *Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR.*

Ketamine, a non-competitive antagonist of N-methyl-d-aspartate (NMDA)-type glutamate receptors, is a commonly used pediatric anesthetic and under certain circumstances, has been shown to be neurotoxic and cardiotoxic in mammals. Reportedly, ketamine decreases heart rate in rats, pigs and infant monkeys. Ketamine has a similar effect on wild type zebrafish (AB strain) larvae that were treated with several concentrations of ketamine (0.5-10 mM): heart rate was decreased in a dose-dependent fashion. After 2 h of exposure, ketamine induced a significant reduction in heart rate in 2-day old larvae at concentrations as low as 1 mM when compared to untreated larvae having 131.6 +/- 5.5 and 152.4 +/- 4.3 beats/min, respectively. However, after 20 h of exposure, 0.5 mM ketamine also reduced heart rate with controls having 186.3 +/- 4.9 and treated, 148.2 +/- 6.8 beats/min. Immunoblot analysis of the lysates of the larvae revealed that ketamine caused significant reductions in activated ERK (MAPK) levels. Interestingly, treatment of the larvae for 2 h with the ERK inhibitor, PD98059, alone also significantly reduced heart rate. Heart rate in ketamine plus PD98059 treated larvae was significantly reduced compared to PD98059 alone, but was comparable to ketamine-treated larvae. Similar treatment with the p38 kinase (MAPK/SAPK family) inhibitor, SB203580, however, did not affect heart rate indicating that ketamine modulated the ERK pathway but not the p38 kinase pathway in slowing zebrafish heart rate. In transgenic (hb9:GFP) embryos, 2 mM ketamine adversely affected motor neuron axon length (20% reduction) and decreased cranial and motor neuron populations (25%) reflected by reduced green fluorescent protein (GFP) expression. These in vivo studies indicate that ketamine adversely affects heart rate and is neurotoxic in zebrafish.
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PS 1615 NEUROCHEMICAL EFFECTS OF MATERNAL HALOPERIDOL EXPOSURE ON THE DEVELOPING RAT.

R. Williams. *Florida A&M University, Tallahassee, FL.*

Antipsychotic drugs are developed primarily to treat major psychiatric illnesses including schizophrenia and the manic phase of polar disorder. Haloperidol is an antipsychotic drug that is frequently prescribed during pregnancy either as an antipsychotic or antiemetic. This experiment investigated the neurochemical effects of maternal haloperidol exposure on developing rat. Animals were kept in a controlled environment of 21 +/- 1 degrees C with the lights on at 07:00 hr and off at 19:00 hr. Dams were randomly assigned to five different groups, i.e., vehicle control; pair fed control (PFC); vehicle control cross fostered; haloperidol cross fostered; and haloperidol. Dams were injected subcutaneously with saline or with 5 mg/kg haloperidol from gestational days 6-20 between 09:00 hr and 12:00 hr. Feed and water were given to PFC dams based on what the haloperidol dams ate the day before. Feed was provided to other controls ab-libitum. After parturition, pups were culled to eight/group, consisting of 4 males and 4 females. Offspring from haloperidol treated dams were cross fostered to nurse from vehicle control dams, and vice versa. At postnatal day (PD) 20 offspring were weaned and separated by sex. On PDs 30 and 100, groups of offspring were sacrificed by decapitation and whole brains removed quickly, weighed and dissected into the caudate nucleus. The caudate nuclei were stored at -70 degrees C until neurochemical analysis. The results obtained showed that maternal doses of haloperidol did not have any significant effect on offspring striatal dopamine D1, D2, or acetylcholine levels. A haloperidol challenge on adult female rats resulted in elevated plasma corticosterone levels compared to plasma basal levels. It was of interest to note that maternal doses of haloperidol did not influence any behavioral parameters tested, except that male offspring on PD 60 showed a decrease in auditory startle response (data not shown).

PS 1616 HISTIOTROPHIC NUTRITION INFORMS DNA METHYLATION IN THE RAT CONCEPTUS.

K. E. Sant, M. Nahar, D. Dolinoy and C. Harris. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Epigenetic processes, such as DNA methylation, regulate the transcription of developmentally-relevant genes that control growth and differentiation during embryogenesis. Methyl groups are enzymatically transferred to 5' sites on cytosine via the methyl donor S-adenosyl methionine (SAM) which is synthesized from methionine using the enzyme methionine adenosyl transferase (MAT2a). Embryonic SAM biosynthesis during organogenesis is dependent on the supply of methionine, vitamin B12, folate, and other methyl cycle components obtained through histiotrophic nutrition pathways. It is hypothesized that disruption of the histiotrophic pathway, through leupeptin inhibition of lysosomal proteolysis, reduces the availability of methyl cycle components, decreasing SAM biosynthesis and resulting in decreased DNA methylation. GD11 rat conceptuses grown in whole embryo culture were exposed to Leupeptin (100μM)for 6 hours. Embryos (EMB) and visceral yolk sacs (VYS) were separated, snap frozen and stored at -80°C prior to DNA isolation and analysis. LUMA assays were used to determine global methylation percentages. In controls, global methylation in VYS (56%) was significantly lower than in EMB (76%)($p<0.01$). Leupeptin treatment caused a significant reduction in global methylation of 6% in EMB and 13% in VYS ($p<0.03$). Pyrosequencing was used to determine site-specific methylation of the MAT2a promoter. MAT2a promoters were methylated at 2% of CG sites in both EMB and VYS and were not affected by treatment. MAT2A specific activities were determined using HPLC detection of SAM. Specific activities were highly variable in the EMB (1952 nM/min/mg prot.) and VYS (266 nM/min/mg prot.) samples ($p<0.05$). Leupeptin did not alter activities in either tissue. This study suggests that disruption of histiotrophic nutrition by protease inhibition decreases global methylation in the EMB and VYS. MAT2 specific activity and site-specific promoter methylation were unchanged by the treatment indicating that no direct effects on the enzyme or its methylation status were responsible for the decrease in global methylation.

PS 1617 A DEVELOPMENTAL TOXICITY STUDY IN SPRAGUE-DAWLEY RATS WITH XOMA 052, A NOVEL MONOCLONAL ANTIBODY TARGETING IL-1 BETA.

K. Meyer[1], C. Gasper[1], J. Ma[1], L. Cao[1], J. Chen[1], K. Der[1] and B. Thorsrud[2]. [1]*Product Development, XOMA (U.S.) LLC, Berkeley, CA and* [2]*Developmental & Reproductive Toxicology, MPI Research, Mattawan, MI.*

XOMA 052 is an ultra-high affinity (300 fM) humanized IgG2 monoclonal antibody that specifically binds to IL-1 beta and inhibits activation of the IL-1 receptor. This activity is expected to prevent the cellular signaling events that produce inflammation. IL-1 beta is a proinflammatory cytokine involved in the development of many diseases including Type 2 diabetes, rheumatoid arthritis, and Behçet's disease. The rat was selected for this developmental toxicity study due to similar binding affinity and *in vitro* functional activity between human and rat IL-1 beta with

XOMA 052. XOMA 052 or the vehicle was administered to pregnant Sprague Dawley rats by subcutaneous injection once weekly on Gestation Day (GD) 6 and 13 at dose levels of 0, 3, 30, and 90 mg/kg. Dams were euthanized on GD 20. Observations and evaluations included clinical signs, gestation body weight and body weight change, food consumption, XOMA 052 serum concentration determination (maternal and fetal), immunogenicity (maternal only), uterine and ovarian examinations, fetal examinations (body weight, sex ratio, external, visceral and skeletal examinations, and total malformations) and maternal macroscopic pathology. All animals survived to scheduled necropsy on GD 20. Results showed no toxicologically significant findings in caesarean section parameters or fetal morphological observations. Serum collected for XOMA 052 concentration analysis on GD 20 from all treated dams and the five tested litters per dose group had similar XOMA 052 concentration levels, demonstrating adequate levels of fetal exposure. Based on these results, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity, including teratogenicity, was considered to be 90 mg/kg, the highest dose level tested. In conclusion, XOMA 052 was well tolerated and exposure did not result in fetal developmental or teratogenic effects when administered to pregnant rats during the period of major organogenesis.

PS 1618 DEVELOPMENTAL HYPOTHYROIDISM DISRUPTS PERFORMANCE OF A SIGNAL DETECTION TASK IN RATS.

M. Hasegawa[1, 2] and H. Wada[1]. [1]*Psychology, Hokkaido University, Sapporo, Japan and* [2]*Japan Society for the Promotion of Science, Tokyo, Japan.* Sponsor: K. Crofton.

Transient hypothyroidism during development of the central nervous system (CNS) impairs cognitive function in rats; however, specific effects on attention are unknown. The effects of transient developmental hypothyroidism on the attention of rats were assessed by utilizing a signal detection task. Pregnant Wistar rats were treated with the anti-thyroid drug, methimazole [2-mercapto-1-methyl imidazole], at concentrations of 0% or 0.02% (w/v) via the drinking water from gestational day 15 to postnatal day (PND) 21 to induce perinatal hypothyroidism. The male offspring were tested on a signal detection task from PND 84. Operant chambers with two response levers and a signal light were used. The signal detection task consisted of signal and non-signal trials. In a signal trial, a brief signal light (500ms, 250ms or 50ms duration) was presented after the inter-trial interval (ITI) elapsed, and subsequently, a limited hold (LH) period for a lever response was initiated. In a non-signal trial, no signal light was presented before the LH period. Rats were required to press the left lever in a non-signal trial and the right lever in a signal trial. Correct responses for signal and non-signal trials were considered as hits and correct rejections (CR), respectively. Hit (%) and CR (%) were calculated to analyze the performance. The total number of lever pressing for left and right levers during the ITI were also monitored. The hit (%) for developmentally hypothyroid rats was significantly decreased from control rats when the durations of the signal light were 250ms and 50ms. The CR (%) and number of left-lever pressing during the ITI were significantly increased for the hypothyroid rats. The decreased hit (%) of the hypothyroid rats during shorter signal durations indicates that perinatal hypothyroidism can impair attention. However, the higher CR (%) and number of left-lever pressing in the hypothyroid rats also suggest the possibility that the decreased hit (%) is due to differences in the response strategies between groups.

PS 1619 PROBING THE TOXCAST™ CHEMICAL LIBRARY FOR PREDICTIVE SIGNATURES OF DEVELOPMENTAL TOXICITY.

N. S. Sipes[1], N. C. Kleinstreuer[1], R. S. Judson[1], D. M. Reif[1], A. V. Singh[3], K. J. Chandler[1, 2], M. R. Rountree[1], D. J. Dix[1], R. J. Kavlock[1] and T. B. Knudsen[1]. [1]*U.S. EPA/ORD/NCCT, Research Triangle Park, NC,* [2]*U.S. EPA/ORD/NHEERL, Research Triangle Park, NC and* [3]*Lockheed Martin, Research Triangle Park, NC.*

EPA's ToxCast™ project is profiling the in vitro bioactivity of chemical compounds to assess pathway-level and cell-based signatures that correlate with observed in vivo toxicity. We hypothesize that cell signaling pathways are primary targets for diverse environmental chemicals that disrupt embryogenesis via combinatorial effects on cellular functions. To test this hypothesis, we built statistical associations based on in vitro high-throughput screening (HTS) data from ToxCast™ and in vivo developmental toxicity data from ToxRefDB. Univariate associations from 2x2 contingency tables were used to filter HTS assays based on statistical correlation with distinct in vivo endpoints. Machine learning algorithms then built predictive models using linear discriminant analysis with multivariate feature selection trained by univariate associations. Initial results gave 389 univariate associations with distinctly different patterns for rat (308 associations) and rabbit (81 associations). Aggregating the species endpoints into a generic model revealed strong correlations between in vitro pathways and in vivo effects for urogenital defects and cleft palate. As an example, a cleft palate signature emerged that included forkhead box (FOX) transcription factor pathways and chromatin remodeling through histone deacetylase (HDAC) signaling. Interestingly, a model built from developmental zebrafish data implied the same target pathway signature for eye reduction defects. Perturbations of these signaling pathways are known to be causally involved in zebrafish craniofacial defects and mammalian (including human) cleft palate. This indicates potential key signaling pathways involved in cleft palate induction may be targets for chemical teratogens, and zebrafish as a model system may reveal similar pathway disruption. This abstract does not necessarily reflect US EPA policy.

PS 1620 *IN OVO* EXPOSURE TO PERFLUOROOCTANOIC ACID DECREASES HATCHING MUSCLE GLYCOGEN IN CHICKEN EMBRYOS.

J. DeWitt[1], I. Bryan[2] and J. Loftis[3]. [1]*Pharmacology and Toxicology, East Carolina University, Greenville, NC,* [2]*Biology and Chemistry, East Carolina University, Greenville, NC and* [3]*Summer Ventures in Science and Mathematics, East Carolina University, Greenville, NC.*

As a result of its use in the manufacture of myriad products, including firefighting foams, stain and water resistant coatings, and fluids for electronics manufacturing, perfluorooctanoic acid (PFOA) is part of the environmental milieu to which organisms are exposed. Studies with laboratory models indicate that PFOA induces metabolic, liver, brain, reproductive, developmental, and immunotoxicities. Of special concern are effects on developing organisms. As PFOA has been reported to affect hatchability in chickens, we evaluated its ability to affect glycogen stores in hatching muscles of developmentally-exposed chicken embryos; glycogen in hatching muscle is necessary for successful hatching. Fertile eggs were air cell injected prior to incubation with 0, 0.5, 1, or 2 mg PFOA/kg egg weight in safflower oil vehicle. After 19 days of incubation, hatching muscles and livers were removed from embryos and immediately frozen. Glycogen was measured in prepared homogenates of each tissue. Liver glycogen levels did not differ by dose. Hatching muscle glycogen levels differed from controls by 43%, 36%, and 30% (0.5, 1, and 2 mg/kg doses, respectively). β-oxidation of fatty acids, which PFOA increases, is most active in the later part of incubation and glucose is derived from yolk fat. Glucose is generated from protein rather than yolk near the end of incubation and is stored in liver and muscles as glycogen (DeOliveira et al., 2008). It is our hypothesis that increased β -oxidation by PFOA during development forces the embryo to utilize protein as energy earlier in development and therefore decreases later glycogen stores, which can affect hatchability and survival. Our data indicate that PFOA decreases hatching muscle glycogen in 19-day-old embryos; additional studies to evaluate glycogen in hatching muscle of hatchlings and at different time points during development are planned.

PS 1621 ARSENIC DELAYS THE MUSCLE DIFFERENTIATION AND REPRESSES MYOGENIN EXPRESSION BY RECRUITING EZH2 AND SUPPRESSING MEF2C IN MOUSE MUSCLE CELLS.

G. Hong and L. Bain. *Biological Sciences, Clemson University, Clemson, SC.*

Arsenic is a toxicant commonly found in water systems around the world. Evidence from epidemiological studies indicates that chronic arsenic exposure can result in cancer, effects on development, and neuromuscular deficits. However, the molecular mechanism of arsenic's toxicity remains largely unclear. Our previous study indicated that 20nM sodium arsenite exposure to C2C12 mouse myocyte cells results in delayed differentiation, caused by a reduction myogenin expression. Repressed myogenin expression was likely due to abnormal DNA methylation in myogenin promoter, which includes two hypermethylated CpGs (-236 and -126) and one hypomethylated CpG (-207). EZH2, which directly silences gene expression via recruiting DNA methyltransferases, is induced by 2-fold in the arsenic treated cells. Additionally, ChIP analysis demonstrated that arsenic exposed cells have a 4-fold increase in the recruitment of EZH2 to the myogenin promoter (-40 to + 42), where contains a MyoD response element and the transcription start site, suggesting that EZH2 may play a regulatory role in myogenin expression.
To further understand the arsenic mediated regulatory mechanisms in muscle differentiation, we are identifying other transcription factors differentially expressed after arsenic exposure. For example, the RNA expression of Mef2c, a transcription factor that is expressed during muscle differentiation, was reduced by 4-fold, while

its nuclear translocation was reduced by 2.7-fold in the arsenic treated cells. We have also found several other transcription factors that interact with the hypomethylated CpG at -207, as well as hypermethylated CpG at -236, using pull down assays. In conclusion, 20nM sodium arsenite exposure to C2C12 cells results in induction of EZH2 and reduction of Mef2C, which may be responsible for the delayed muscle differentiation. Additionally, the hypermethylated CpG at -236 and the hypomethylated CpG at -207 in myogenin promoter may also play a role in myogenin expression by blocking the binding of transcription factors (or repressors) to these CpGs.

PS 1622 EFFECTS OF LOW-DOSE PRENATAL PFOA EXPOSURE ON THE MAMMARY GLAND OF CD-1 MICE.

M. B. Macon[1, 2], L. R. Villanueva[3, 4], R. D. Zehr[5], K. Tatum-Gibbs[1, 4], M. J. Strynar[5], J. P. Stanko[2], S. S. White[2], L. Helfant[5] and S. E. Fenton[2]. *[1]Toxicology, University of North Carolina, Chapel Hill, NC, [2]NTP, NIEHS, Research Triangle Park, NC, [3]Chemistry, North Carolina Central University, Durham, NC, [4]TAD, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC and [5]HEASD, NERL, ORD, U.S. EPA, Research Triangle Park, NC.*

Perfluorooctanoic acid (PFOA) is an environmental contaminant that has been shown to delay mammary gland (MG) development in prenatally exposed mice. The effects of PFOA on MG development were investigated at varying low doses and exposure lengths. Timed-pregnant CD-1 mice were gavaged with 0, 0.3, 1.0, and 3.0 mg PFOA/kg body weight (BW) daily from gestation days (GD)1-17 or 0, 0.01, 0.1, and 1.0 mg PFOA/kg BW daily from GD10-17. MGs, liver, and trunk blood were collected from offspring on postnatal day (PND) 7, 14, 21, 28, 42, 63, and 84 in the GD1-17 exposure and on PND 1, 4, 7, 14, and 21 in the GD10-17 exposure. Serum was analyzed for PFOA concentration using HPLC-MS/MS. MGs were prepared as whole mounts, histologically evaluated for growth hallmarks, and assigned developmental scores on a scale of 1-4 (1=least developed; 4=normal/best developed). MGs of offspring exposed on GD10-17 were also evaluated for quantitative characteristics. When compared to controls, all PFOA-treated groups displayed signs of aberrant MG growth. These effects were significant from as early as PND7 to PND84 in the GD1-17 exposure and at PND21 in the GD10-17 exposure study. MGs of the 1.0 mg/kg groups exposed for GD10-17 were smaller in size, had less longitudinal epithelial growth, and fewer terminal end buds relative to controls. PFOA serum concentrations remained elevated for up to six weeks and was detected in the brain for up to 4 weeks. Across time, serum levels were highest at PND 14 in offspring of dams exposed from GD1-17. Hepatomegaly was evident in all GD1-17 PFOA exposure groups and in the 1.0 mg/kg group of the GD10-17 exposure study. These findings suggest that lowest observable adverse effect level (LOAEL) of prenatal PFOA exposure on liver weight is 0.03 mg/kg and the LOAEL on the MG is 0.01 mg/kg. However, the persistence of the MG effect is unclear. This abstract does not necessarily reflect NIEHS or EPA policy.

PS 1623 PYRIMETHAMINE – INVESTIGATIVE EMBRYO-FETAL DEVELOPMENT STUDY BY THE ORAL ROUTE (GAVAGE) IN THE MINIPIG WITH MID-TERM CAESAREAN SECTIONS.

E. C. Marsden, C. Pique and P. C. Barrow. *Ricerca Biosciences SAS, Lyon, France.*

The minipig may be ignored as an alternative non-rodent species for embryo-fetal development studies for reasons of cost and study duration. The principal objective of our study was to evaluate if fetal abnormalities associated with a known teratogen, pyrimethamine, could be detected in the minipig if fetal examinations are performed mid-term (close to gestation day (GD) 60) compared with current standard examinations at term (close to GD 110). Thirty six 6 to 8 month old mated Göttingen Minipig gilts were divided into two groups of 18 females each; one was administered pyrimethamine by daily gavage at 3.6 mg/kg/day and the other received the vehicle (1 % CMC). Each group was further divided into two subgroups of 9 minipigs each and underwent either mid-term or term caesarean section. The females were dosed throughout the major period of organogenesis (GD's 11 to 35). Maternal clinical condition and body weight were monitored throughout the study. At necropsy, the females were examined macroscopically, litter parameters were recorded and fetuses were weighed and sexed. The fetuses (mid-term and term) were initially examined for external and visceral abnormalities and then processed for skeletal examination. There were no remarkable compound-related maternal effects. At scheduled caesarean section, 16 and 18 females were pregnant in the control and pyrimethamine groups, respectively. Although a low number of viable litters was available due to a high incidence of compound-related embryo-fetal death, fetal abnormalities consistent with those described in the literature, principally limb malformations, micrognathia and cleft palate, were detected at the mid-term examinations. We conclude that mid-term fetal examinations are feasible technically, provide numerous practical and time saving advantages with respect to term caesareans and thus provide a realistic alternative for the testing of small molecules, ultimately reducing study duration and associated costs.

PS 1624 WNT INHIBITORY FACTOR 1 (WIF1) PROMOTES PROSTATIC BUD FORMATION AND MAY PARTIALLY PROTECT AGAINST DEFECTS IN PROSTATE DEVELOPMENT CAUSED BY TCDD EXPOSURE.

A. Branam[1], R. W. Moore[1], L. L. Abler[2], S. H. Allgeier[1], V. Mehta[2], C. M. Vezina[2] and R. E. Peterson[1]. *[1]School of Pharmacy, University of Wisconsin, Madison, WI and [2]Comparative Biosciences, University of Wisconsin, Madison, WI.*

Prostatic buds are derived from the urogenital sinus (UGS) and later form into prostate ducts in adult mammals. In utero TCDD exposure causes dorsal and lateral prostatic buds in male mice to form in inappropriate positions and prevents formation of ventral and some dorsal prostatic buds. Wnt signaling has been implicated as having important roles in prostatic bud formation. Our previous results showed that in vitro treatment of male UGSs with either TCDD or WNT5A decreased prostatic bud formation, and that antibody against WNT5A rescued the effects caused by TCDD. We propose that TCDD causes an imbalance in Wnt signaling leading to defects in prostate development, and we hypothesize that extracellular antagonists of Wnt signaling can rescue the effects of TCDD via a similar mechanism as the WNT5A antibody by preventing WNT5A from binding its receptors and thus inhibiting its effects. C57BL/6J dams were dosed with TCDD (5 μg/kg, po) or vehicle on e15.5 and UGSs were harvested on e16.5. Sectional ISH analysis using probes directed against genes for the Wnt antagonists (Sfrp2, Sfrp3, Dkk1, Dkk2, and Wif1) were performed to look for differences in expression patterns between vehicle- and TCDD-treated UGSs. Wif1 was the only gene examined that showed a response to TCDD, a decrease in expression. RT-PCR confirmed these results. This effect is seen in the ventral UGS, the site most affected by TCDD exposure. Furthermore, addition of WIF1 to in vitro UGS cultures containing DHT increased the number of prostatic buds formed as compared to control UGSs treated with DHT alone. Preliminary evidence suggests that addition of WIF1 may partially protect against the effects of TCDD in culture. These results suggest that WIF1 may be a key modulator of prostatic bud formation and that addition of WIF1 may reduce the effects of TCDD exposure on prostate development. (Grant support: ES01332, T32 ES007015, DK083425)

PS 1625 EFFECTS OF PERFLUORINATED PHOSPHONIC ACID EXPOSURE DURING PREGNANCY IN THE MOUSE.

K. R. Tatum-Gibbs[1], K. P. Das[2], B. Grey[2], M. Strynar[3], A. Lindstrom[3] and C. Lau[2]. *[1]Curriculum in Toxicology, University of North Carolina-Chapel Hill, Research Triangle Park, NC, [2]Developmental Toxicology Branch, U.S. EPA -RTP, Research Triangle Park, NC and [3]HEASD, U.S. EPA -RTP, Research Triangle Park, NC.*

Perfluorinated phosphonic acids (PFPAs) are a member of the perfluoroalkyl acid (PFAA) family, and are structurally similar to the perfluoroalkyl sulfonates and perfluoroalkyl carboxylates. These chemicals have recently been detected in the environment, particularly in surface water and in effluent of wastewater treatment plants. PFPAs are used primarily as a surfactant defoaming agent in the textile industry and in pesticide production. Previous studies from our laboratory have identified developmental toxicity associated with gestational exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), but little is known about this emerging class of perfluorinated chemicals. Therefore, the current study examined the developmental effects of PFPA in the mouse. A mixture of PFPAs (Masurf-780) was given to timed-pregnant CD-1 mice by oral gavage daily throughout gestation (GD 1-17) at doses of 5, 10, 20, 30 or 40 mg/kg; controls received deionized water vehicle. PFPA did not alter maternal weight gain, but significantly increased maternal liver weight at term (GD-17) at all dose groups. Recovery in maternal liver weight was observed by weaning (PND 21). Chemical exposure did not influence the number of live fetuses or fetus weights at GD-17, except in the 40 mg/kg group where mortality was observed. In contrast, fetal liver weight was significantly increased at doses greater than 5 mg/kg. Neonatal survival and growth was monitored on postnatal days 1-42 and were generally not altered except in the 40 mg/kg group, where the increase in neonatal liver weight persisted. These data suggest that gestational exposure to PFPA at doses less than 40 mg/kg

does not compromise neonatal survival and postnatal growth as seen with PFOS and PFOA, while the hepatic effects appear to be similar among these chemicals. This abstract does not necessarily reflect U.S. EPA policy.

PS 1626 IDENTIFICATION OF *Wnt* AND *Rspo* GENES WHOSE EXPRESSION PATTERNS IN FETAL MOUSE UROGENITAL SINUS (UGS) ARE ALTERED BY 2, 3, 7, 8-TETRACHLORODIBENZO- *p* -DIOXIN (TCDD).

R. W. Moore[1], L. L. Abler[2], V. Mehta[2], C. M. Vezina[2] and R. E. Peterson[1]. [1]*School of Pharmacy, University of Wisconsin, Madison, WI and* [2]*Comparative Biosciences, University of Wisconsin, Madison, WI.*

Wnt genes regulate tissue patterning, cell fate, and proliferation during embryogenesis, but little is known about their role in UGS and prostate development. Our lab is testing the hypothesis that *in utero* TCDD exposure alters prostatic budding patterns and reduces prostatic bud number by dysregulating Wnt signaling. Specific objectives were to determine the expression patterns in control UGSs of the 19 known mouse *Wnt* genes, and to determine if TCDD changes these patterns. Because canonical Wnt signaling is also activated by R-spondins, expression of the four *Rspo* genes was also examined. Pregnant C57BL/6J mice were dosed with TCDD (5 μg/kg, po) or vehicle on e15.5, and UGSs were collected 24 hr later. Gene expression patterns were determined by *in situ* hybridization on sagittal UGS sections. Expression of *Wnt1, Wnt3, Wnt8a, Wnt8b*, and *Wnt10b* was very low or absent in both vehicle- and TCDD-exposed UGSs. *Wnt2a, Wnt2b, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, Wnt9a, Wnt9b, Wnt11, Rspo1, Rspo3*, and *Rspo4* were expressed in diverse patterns, but TCDD had little if any effect on any of these patterns or their expression intensities. TCDD had no detectable effect on where *Wnt7b* was expressed (throughout the epithelium except in basal urothelium), but it appeared to increase expression intensity. Expression of *Wnt10a* was generally confined to the basal epithelium, but TCDD exposure enhanced this expression in the ventral prostatic budding region (where budding inhibition is greatest). *Wnt16* was expressed primarily in pelvic urethral mesenchyme in control UGSs, but TCDD extended its expression into the ventral UGS mesenchyme. TCDD reduced *Rspo2* expression in ventral UGS mesenchyme. Future research will investigate whether altered expression of these four genes contributes to the prostatic budding abnormalities caused by TCDD. (Supported by NIH grants ES01332 and DK083425.)

PS 1627 ZEBRAFISH EMBRYOS SEQUESTER PETROCHEMICAL COMBUSTION PRODUCTS IN LIPID DROPLETS, WITH UP-REGULATION OF BIOTRANSFORMATION, OXIDATIVE STRESS, AND INFLAMMATION-RELATED GENES.

R. Xiao, A. Bui, K. Kleinow and A. Penn. *Louisiana State University School of Veterinary Medicine, Baton Rouge, LA.*

Rationale: Numerous fluorescent polynuclear aromatic hydrocarbons (PAHs) are adsorbed onto the surface of nanoparticles produced during incomplete combustion of petrochemicals. Incomplete combustion of the high volume petrochemical 1,3-butadiene (BD) yields PAH-rich soot (BDS) nanoparticles. Inhalation of these particles results in up-regulation of biotransformation, oxidative stress and inflammatory genes in mouse lungs. The zebrafish (ZF) embryo is a well-established model for vertebrate development and is sensitive to exposure to a range of environmental toxicants. We use ZF embryos to examine cell/molecular responses to PAHs early in development. ZF embryos exposed to BDS nanoparticles fluoresce and exhibit developmental abnormalities. Our goals here were to a) determine where the PAH fluorescence is localized in ZF embryos and b) assess patterns of embryo gene expression responses to BDS exposure. Methods: We exposed ZF embryos to BDS nanoparticles (0,6,60 microg/ml) 24-72 hr. post-fertilization. We assessed images, singly and merged, of Nile Red and BDS fluorescence; performed trranscriptome analysis of pooled embryos (Affymetrix ZF Arrays; Expression Analysis; Ingenuity Pathways Analysis); and confirmed results by qRT-PCR. Results: We found 49 unique genes with absolute fold-change >1.5X in both of the treated vs control group comparisons. Those genes were displayed on a heatmap (R/Bioconductor) and cross-tabulated by biofunction (Ingenuity). Nile Red and PAH fluorescence co-localized in lipid droplets. PAH fluorescence and up-regulation of ZF genes homologous to human biotransformation, oxidative stress and acute phase response genes proceeded in a dose-dependent manner. Conclusion: ZF embryos reprise a number of mammalian responses to petrochemical combustion-derived PAHs. ZF embryos may be valuable for assessment of developmental responses to PAHs arising from combustion-based remediation of the BP Horizon oil spill.

PS 1628 PRENATAL TCDD AND POSTNATAL AUTOIMMUNE DISEASE: A COMPARISON OF TWO MURINE STRAINS.

S. D. Holladay, A. Mustafa and R. M. Gogal. *Department of Anatomy and Radiology, University of Georgia, Athens, GA.*

Pregnant female C57BL/6 and SNF1 mice were exposed to a mid-gestation dose of 2,3,7,8-tetrachlrordibenzo-p-dioxin (TCDD) and the offspring evaluated at adulthood. The C57BL/6 mouse, a non autoimmune-prone strain, has a high-affinity aryl hydrocarbon receptor (AhR) making it sensitive to TCDD where as, the SNF1 mouse has a low-affinity AhR and spontaneously develops autoimmunenephritis. At 24 weeks, prenatal TCDD-exposed C57BL/6 mice had increased autoreactive Vβ+CD4+17a and Vβ+CD3+ T cells biased toward the females. Cytokine levels fluctuated during aging yet IFN-γ was elevated in the females, while IL-10 was elevated in males at 48 weeks. In these prenatal TCDD C57BL/6 mice, B-lineage cells were altered in the bone marrow and spleen, and circulating autoantibodies were increased. In these same mice, there was significant anti-IgG and anti-C3 renal deposition, suggesting an early stage of autoimmune lupus. The prenatal TCDD SNF1 offspring similarly showed increased peripheral Vβ+ cells and increased IFN-γ production in the females plus increased autoantibody production in both sexes. Likewise, male prenatal TCDD SNF1 mice had increased anti-IgG and anti-C3 deposition in kidneys suggesting an earlier onset of lupus. Both mouse models therefore showed clear signatures of enhanced autoimmunity and autoimmune disease after prenatal TCDD.

PS 1629 EFFECT OF LOW-DOSE MERCURIC CHLORIDE EXPOSURE ON EARLY ZEBRAFISH EMBRYO DEVELOPMENT.

L. C. Abbott[1], E. A. Moussa[2] and S. A. Hassan[3]. [1]*Integrative Biosciences, Texas A&M University, College Station, TX,* [2]*Anatomy and Embryology, Suez Canal University, Ismailia, Egypt and* [3]*Anatomy and Embryology, Suez Canal University, Ismailia, Egypt.*

Much attention has focused on environmental contamination by heavy metals, pesticides, and polychlorinated biphenyls. Mercury has been reported as a ubiquitous environmental pollutant that is able to accumulate in the aquatic food chain with severe risk for animal and human health. Zebrafish (Danio rerio) provide an excellent vertebrate animal model system to study mechanisms of toxicity. Starting at 6 h post fertilization, zebrafish embryos were exposed for 42 hours to different concentrations of mercuric chloride (HgCl): 0 [negative control], 100, 200, 300 and 400, 500, 1000 and 1500 ppb. Zebrafish embryos exposed to 2% ethanol served as positive controls (100% embryonic death). Embryos were assessed at 48h post fertilization for progress in development, morphometry and morphological deformities of the trunk musculature. Immunohistochemical staining of muscle using antibody to myosin was carried out on 5 micron thick sections of zebrafish embryos that were fixed with 4% paraformaldehyde and embedded in paraffin. Embryos exposed to 100 ppb HgCl revealed abnormal myosin staining (irregular diameter of muscle segments and decreased intensity of myosin staining) compared to control zebrafish embryos. Zebrafish embryos exposed to 200 and 300 ppb HgCl presented irregularly stained bands of trunk musculature. Embryos exposed to 400 ppb HgCl showed no staining for myosin indicating a significant delay in development of myosin expression. Trunk and tail muscle fibers from additional paraffin sections from zebrafish embryos exposed to 100 through 400 ppb HgCl were stained with eosin and analyzed for morphologic parameters, including muscle fiber length and degree of waviness. In general, abnormalities increased with increased exposure to HgCl. These data indicate that even low dose exposure to HgCL can delay or alter normal development of trunk musculature in exposed zebrafish embryos.
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PS 1630 DEVELOPMENTAL TOXICITY OF THE HMG-COA REDUCTASE INHIBITOR (PPD10558) IN RATS AND RABBITS.

A. S. Faqi[1], D. Prohaska[2], R. Lopez[2] and G. McIntyre[2]. [1]*Drug Safety Evaluation, MPI Research, Mattawan, MI and* [2]*Drug Safety Evaluation, Furiex Pharmaceuticals, Morrisville, NC.*

PPD10558 is an orally active, lipid-lowering 3 hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor (Statin) being developed as an anti-cholesterol drug. We have studied the potential developmental toxicity effects of PPD10558 in pregnant rats and rabbits given daily oral doses during the period of organogenesis. Rats were dosed with 0, 20, 80 or 320 mg/kg/day from GD 6 to 17 and rabbits received dose levels of 0, 12.5, 25 or 50 mg/kg/day from GD 6 to 18. Additional groups in both studies served as TK animals and received the vehicle or PPD10558 in the same manner as the main study groups at the same dose levels. Blood samples

were collected from TK animals at designated time points on GD 6 and 17 in rats and GD 6 and 18 in rabbits. On GD 20 or GD 29 maternal and developmental parameters were evaluated and in rats, fetal exposure was assessed on GD 20. No maternal and developmental toxicity was observed at any of the dose levels used in the rat study. Evidence of fetal exposure was determined in fetal plasma with mean fetal concentrations of PPD10558 and the metabolite (PPD11901) found to be between 1% and 6% of the mean maternal concentrations. In rabbits, marked maternal toxicity including mortality (8 deaths; 1 doe at 25 and 7 at 50 mg/kg/day), abortions (2 at 25 mg/kg/day and 6 at 50 mg/kg/day) and reduction in gestation body weight, gestation body weight changes and decreased food consumption were observed. In addition, fetal body weights of the combined sexes were significantly reduced at 50 mg/kg/day in comparison to the controls. Based on a human dose of 80 mg BID the margins of exposure for teratogenic effects are at least 19x and 6x for rats and rabbits, respectively, based on the top doses of 320 mg/kg/day for rats and 50 mg/kg/day for rabbits. In conclusion, these studies show no evidence of teratogenicity of PPD10558 in both rats and rabbits, though overt maternal toxicity occurred in rabbits.

PS 1631 FOXQ1: A BRIDGE BETWEEN THE AHR AND HEDGEHOG SIGNALING PATHWAYS.

A. Planchart. *Mount Desert Island Biological Laboratory, Salisbury Cove, ME.*

The aryl hydrocarbon receptor (AHR) pathway has been of particular interest in studying the effects of environmental exposures on development because this pathway functions as an "antennae", receiving input from the external environment that leads to changes in gene expression. Toxicant activation of the AHR pathway leads to craniofacial defects in model organisms including the zebrafish and mouse that are similar in pathology to some human craniofacial abnormalities. The hedgehog signaling pathway, a critical pathway required for the development of the vertebrate head and face and implicated in several human craniofacial disorders, is misregulated as a result of AHR pathway activation yet how this occurs is currently unknown. Our lab was the first to identify foxq1l, an evolutionarily conserved forkhead box transcription factor in zebrafish, as a target of the dioxin-activated AHR pathway. Foxq1l is activated by dioxin exposure in the zebrafish head, particularly in embryonic structures that give rise to the upper and lower jaw. We hypothesized that the AHR pathway links environmental exposures to craniofacial birth defects by misregulating the hedgehog signaling pathway via an intermediary signaling molecule, possibly foxq1l. We now demonstrate that misexpressing foxq1l leads to misregulation of the hedgehog pathway and abnormal craniofacial chondrogenesis, and consequently to abnormal head development in affected embryos, supporting the hypothesis that foxq1l functions as a bridge between the AHR and hedgehog signaling pathways. We propose that this mechanism is conserved in all vertebrates.

PS 1632 CLEFT PALATE IN RATS TREATED WITH A COMPOUND THAT HAS STEROL ANTISYNTHESIS ACTION.

N. Ikemi[1], T. Oota[2] and K. Kawashima[2]. *[1]Otsuka AgriTechno Co., Ltd., Tokushima, Japan and [2]Mitsubishi Chemical Medience Co., Ltd., Kumamoto, Japan.*

[Objective] We have been conducting experiments to investigate the mode of occurrence of cleft palate. The present experiments were designed to specify the period of sensitivity to a compound with a sterol synthesis inhibition action in induction of cleft palate in SD rats and to determine whether induction of cleft palate was due to the parent compound or its metabolite following amniotic fluid administration of this compound.
[Methods] (Experiment 1) To identify its cleft palate induction action, forced oral administration of the compound to the animals was conducted once daily for 10 days from the 6th day to 15th day of pregnancy (vaginal plug confirmation day: day 0 of pregnancy). The doses were set at 15, 20 and 25 mg/kg. The animals underwent cesarean section on day 20 of pregnancy. (Experiment 2) To identify the critical period for the occurrence of cleft palate, a single dose (100 mg/kg) of the compound was administered to dams, and the animals underwent cesarean section on day 20 of pregnancy. (Experiment 3) To investigate whether the induction of cleft palate following administration of the compound was caused by the compound itself or by its metabolite, the abdomens of the dams were incised, and the compound was administered into the amniotic fluid through the uterine wall. Results] (Experiment 1) The incidences of cleft palate were 11%, 43% and 76% in the 15, 20 and 25 mg/kg groups, respectively. (Experiment 2) The occurrence of cleft palate was observed following administration of every single dose on days 10, 11, 12 and 13 of gestation. Based on a comparison of the incidences of cleft palate, the critical period for the occurrence of cleft palate was considered to be the day 13 of gestation. (Experiment 3) No cleft palate was observed. [Conclusion] It was possible that metabolites of the compound induced cleft palate as cleft palate occurred frequently in fetuses of dams receiving an oral dose of the compound, while it was not observed in fetuses given the compound via the intraamniotic route.

PS 1633 CHEMICAL FORM MATTERS: DIFFERENTIAL ACCUMULATION OF ORGANIC AND INORGANIC MERCURY IN ZEBRAFISH LARVAE.

T. C. MacDonald, M. Korbas, I. J. Pickering, G. N. George and P. H. Krone. *University of Saskatchewan, Saskatoon, SK, Canada.*

Mercury is found in both organic and inorganic forms in the atmosphere, in sediment and in water bodies due to natural and anthropogenic sources. Limited information is available on the uptake and accumulation of Hg in developing organisms, particularly as it relates to chemical form of the metal. To address this problem we utilized synchrotron x-ray fluorescence imaging in zebrafish (Danio rerio), an increasingly well studied model vertebrate for investigating mechanisms of chemical toxicity. Zebrafish larvae were exposed to one of four forms of mercury (methyl mercury chloride, methyl mercury L-cysteine, mercuric chloride, and mercury bis-L-cysteineate). Adjacent serial sections were utilized for synchrotron imaging and histological staining, respectively. Sections were imaged using the X-ray fluorescence imaging technique on beamline 20-ID at the Advanced Photon Source (APS) in Argonne, IL. Significant variations in mercury accumulation were found in fish exposed to organic mercury as compared to fish exposed to inorganic mercury. Larvae exposed to inorganic mercury exhibited accumulation in the ventricular region of the brain. However, only the fish exposed to organic mercury exhibited accumulation in the lens epithelium, gut tube, and skeletal muscles. These variations demonstrate the importance of considering chemical form when considering the uptake and accumulation of mercury. Ongoing research will investigate the efficacy of chelating agents. Although dimercaptosuccinic acid (DMSA) is not a true chelator it is currently used to treat mercury exposure. As DMSA is poorly optimized for this role it is not always effective. Other sequestration agents of interest are N-acetylcysteine (NAC) and alpha lipoic acid (ALA). Eventually, we hope to design and test a custom chelator that can bind and excrete different forms of mercury more effectively.

PS 1634 ABERRANT LIGAND-INDUCED ACTIVATION OF G-PROTEIN-COUPLED ESTROGEN RECEPTOR 1 (GPER) RESULTS IN DEVELOPMENTAL MALFORMATIONS DURING VERTEBRATE EMBRYOGENESIS.

B. S. Jayasinghe and D. C. Volz. *Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC.*

G-protein-coupled estrogen receptor 1 (GPER) – a GPCR unrelated to nuclear estrogen receptors (ERs) but strongly activated by estradiol in both mammals and fish – is expressed and functional in numerous vertebrate organ systems and has been implicated as a potential target for xenoestrogen-mediated signaling in reproductive organs and tumors. To date, the distribution and functional characterization of GPER within vertebrate organs have been restricted to juvenile and adult animals. In contrast, virtually nothing is known about the spatiotemporal distribution and function of GPER during vertebrate embryogenesis. Using zebrafish as an animal model, the aim of this study was to assess the functional role of GPER during embryogenesis. RT-PCR and whole mount in situ hybridization data suggest that GPER mRNA is expressed as early as 1-hr post-fertilization (hpf) and is strongly localized in the central nervous system. Continuous exposure to a selective GPER agonist (G-1) from 8 to 96 hpf resulted in dose-dependent effects on survival and gross larval morphology, including decreased body length, tail abnormalities, and axial curvature. However, exposure to a selective GPER antagonist (G-15) resulted in no discernible effects on survival or gross larval morphology. Importantly, based on co-exposure studies with G-1 and G-15, G-15 fully blocked G-1-induced morphologic effects, suggesting that G-1-induced effects are mediated via aberrant activation of GPER. Using reverse genetics (morpholino-based) strategies, we are currently testing whether (1) GPER knockdown alone results in developmental abnormalities and (2) translation of GPER (or other ERs) is required for G-1-induced effects on gross larval morphology. Overall, our findings suggest that aberrant ligand-induced GPER activation represents a potentially novel and understudied mode-of-action for environmentally relevant chemicals that affect vertebrate embryogenesis.

PS 1635 PRENATAL BISPHENOL-A ALTERS LUNG EPITHELIAL SECRETORY CELL MATURATION.

S. Murphy, M. Boetticher, C. VandeVoort and L. Van Winkle. *California National Primate Research Center, UC Davis, Davis, CA.*

Bisphenol-A (BPA) is used in the production of polycarbonate plastics. We hypothesized that exposure to BPA can disrupt fetal lung development because both airway and alveolar development occur in the prenatal period. To test this hypothesis we exposed timed pregnant Rhesus macaques to BPA. There were 3 exposure

groups 1) Sham control early term(CO-E), 2) Sham control late term (CO-L), 3) BPA early term (BPA-E) and 4) BPA late term (BPA-L). BPA exposed animals received subcutaneous BPA in two doses 4 weeks apart and lungs were sampled at 100 days gestational age (DGA) or 150DGA. The distribution and abundance of secretory proteins was defined using immunohistochemistry for Clara cell secretory protein (CCSP) and pro-surfactant protein C (SP-C). Histologic staining with Alcian Blue-Periodic Acid Schiff (AB-PAS) identified mucous containing cells. Further we quantified gene expression of SPC and CCSP in microdissected lung subcompartments from the same animals. CCSP protein was increased in proximal airways in the BPA-L group; more cells contained CCSP and the cells that contained it had more CCSP per cell. There was a 3 fold increase in CCSP gene expression in the proximal airways of the BPA-L treated animals compared to age CO-L. Mucous cells in the large conducting airways were increased in the BPA-L group. In the alveoli, BPA exposure increased the expression of pro-SP-C in both the BPA-E and BPA-L groups compared to CO. BPA exposure induced a dichotomous response in the alveoli depending on the timing of the exposure. In the BPA-E group there was an approximately 2.5 fold increase in SPC expression. The trend in the BPA-L group was a slight decrease in SPC compared to CO-L. We conclude 1) that conducting airway secretory cell maturation is accelerated by BPA exposure when it occurs late in gestation but not early, 2) that alveolar type II cell secretory product maturation is also affected by BPA exposure both early and late in gestation and 3) the timing of exposure also influences the nature and extent of the response. Support: ES016770, ES016249 and ES000628

PS 1636 ESTIMATED DIETARY EXPOSURE AND PREDICTORS OF SERUM CONCENTRATIONS OF PERFLUORINATED COMPOUNDS IN 119 PREGNANT NORWEGIAN WOMEN.

A. Brantsaeter, M. Haugen, L. S. Haug, H. K. Knutsen, H. E. Kvalem, C. Thomsen, G. Becher, J. Alexander and H. Meltzer. *Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway.* Sponsor: M. Løvik.

Diet, and seafood in particular, has been shown to be an important source of perfluorinated compounds (PFCs) in humans. The aim of the present study was to identify predictors of serum perfluorooctane sulfonic (PFOS) and perfluorooctanoic acid (PFOA) concentrations in 119 pregnant women participating in a validation study within the Norwegian Mother and Child Cohort Study (MoBa). Blood mercury and serum concentrations of 19 PFCs were analysed, and information about habitual dietary intake, demographic and sociodemographic factors were given by self-report around gestational week 20. The mean estimated dietary exposure to PFOS and PFOA was 55.1 and 36.6 ng/kg bw/day, and mean serum concentrations were 16.6 ng/ml and 2.9 ng/ml respectively. Predictors of PFOS and PFOA concentrations were evaluated using multiple linear regression analysis, adjusting for dietary intake. The following predictors were identified; for each month of previous breastfeeding the concentration of PFOS was lowered by 1.85% [β=-0.30 (95% CI: -0.40, -0.19)] and of PFOA by 1.75% [β=-0.07 (95% CI: -0.09, -0.05)]. Blood Hg (a marker of seafood consumption) was associated with an increase in the concentration of PFOS with 7.3% and of PFOA with 5.3%. Current smoking was associated with reduced PFOS, but not PFOA concentration. Age and body mass index influenced these PFCs but did not reach statistical significance. MoBa, which in the years 1999 to 2008 has included more than 108 000 pregnant women, offers a unique opportunity for investigating potential health effects of pre- and postnatal exposure to these compounds. The children are followed up through questionnaires, clinical sub-studies and national registries. In future studies we plan to use predicative models to identify infants having high exposure to PFCs in utero and during early life and to relate the exposures to physical and neurodevelopmental outcomes.

PS 1637 EFFECTS OF PCBS ON EMBRYONIC CARDIAC DEVELOPMENT IN GALLUS (*GALLUS DOMESTICUS*).

T. Carro and M. Ottinger. *Animal and Avian Sciences, University of Maryland, College Park, MD.* Sponsor: R. Schuh.

Polychlorinated biphenyls (PCBs) are a class of synthetically made compounds that have been linked to a variety of adverse health conditions. Previous studies have suggested a link between exposure to PCBs in mammalian and avian species and adverse cardiovascular effects, especially in high-risk populations, such as organisms in the developmental stages of life. The purpose of this study was to determine the effects of exposure to an environmentally relevant mixture of PCBs on embryonic hatch success, embryonic heart function, and embryonic heart development in a model species, Gallus gallus domesticus (domestic chicken). Chicken eggs were prepared by drilling a small hole into the side of the shell; treatments were administered via micropipette injection directly into the albumen and then sealed with paraffin wax. Twenty eggs per treatment group were randomly assigned as follows: untreated control, sham injection, 0.0 μg/g egg wt, 0.03 μg/g egg wt, 0.08 μg/g egg wt, 0.3 μg/g egg wt, 0.5 μg/g egg wt, 0.7 μg/g egg wt, and 2.06 μg/g egg wt (total volume administered was less than 1.5 μl/egg). Embryo viability was monitored by candling at four day intervals throughout development; heart rate was measured by external detection of heart beat during the last week of incubation. Chicks were necropsied at hatch; hearts were immediately collected, weighed, and uniformly fixed in 10% buffered formalin. Hearts were embedded in paraplast wax, transversely sectioned, and stained with hematoxylin and eosin. Heart morphology was analyzed at four layers, using heart structures to establish uniformity across sections. Results showed dose-related effects between the concentrations of PCBs, heart rates, and hatch success. No significant differences were detected in organ indices or body weight. Heart morphology showed gross cardiomyopathies in PCB treated hatchling hearts. These results provide supporting evidence to deleterious effects of embryonic exposure to PCBs.

PS 1638 POLYCYCLIC AROMATIC HYDROCARBONS EXERT STRUCTURE-DEPENDENT DIFFERENTIAL MECHANISMS OF TOXICITY IN DEVELOPING ZEBRAFISH.

B. C. Goodale[1], J. K. La Du[1], S. C. Tilton[2], K. M. Waters[2] and R. L. Tanguay[1]. [1]*Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR and* [2]*Pacific Northwest National Laboratory, Richland, WA.*

While polycyclic aromatic hydrocarbons (PAHs) are well-known for their carcinogenic properties, recent studies have drawn attention to other PAH-induced human health effects including cardiac and developmental toxicity. These alternate toxicological mechanisms are of particular concern as fossil fuel combustion and accidental spills increase levels of PAHs in the environment. Studies in aquatic organisms indicate that PAHs induce differential structure-dependent effects during development. Many 4-5 ring PAHs activate the aryl hydrocarbon receptor (AHR), which regulates expression of a number of responsive genes including CYP1A, and induce AHR- and CYP1A-dependent developmental effects. Other PAHs induce toxicity, such as cardiac malformations, via AHR-independent mechanisms. Understanding the mechanisms by which structurally-similar groups of PAHs induce toxicity is essential for evaluating potential health risks associated with environmental exposure to these compounds. In this study, zebrafish were exposed to the model PAHs pyrene, benz(a)anthracene and dibenzothiophene at 6 hours post fertilization (hpf) and assessments were conducted to establish concentrations that induce morphological effects but not mortality at 120 hpf. These 3-4 ring PAHs induce distinct biological responses in zebrafish that are AHR2 and CYP1A dependent, AHR2 dependent, and AHR2 independent, respectively. Whole animal mRNA microarray analysis was conducted at 24 and 48 hpf to compare mRNA expression levels in PAH and vehicle control-exposed zebrafish embryos. Comparative analysis identified differential mRNA expression between exposure groups, which was further analyzed to uncover regulatory networks of PAH-induced toxicity. These data will inform future studies aimed at elucidating the multiple mechanisms by which PAH structures disrupt developmental processes. This research is supported by NIEHS grants P30ES00210, P42ES016465 and T32ES07060.

PS 1639 GENE EXPRESSION EVALUATION OF THE RELATIVE SAFETY OF PRENATAL STRESS AND/OR ANTIDEPRESSANT EXPOSURE.

C. H. Bourke, C. F. Capello and M. J. Owens. *Psychiatry and Behavioral Sciences, Emory University, Atlanta, GA.*

Pregnancy expands a woman's health considerations beyond herself to include her unborn child. In the case of a woman diagnosed with major depressive disorder, drug intervention may be the only effective treatment. While most clinical studies show that intervention with an SSRI is generally safe during pregnancy, long-term effects on the infant are unknown. The purpose of this study is to evaluate the effects of these prenatal treatments on the offspring's gene expression in adulthood. Female rats were implanted with osmotic minipumps that delivered clinically relevant concentrations of the SSRI escitalopram throughout the entire course of pregnancy. After mating, females were exposed to a chronic unpredictable mild stress model that putatively recapitulates aspects of clinical depression. The offspring were divided into four groups based on their in utero environment: control, stress, escitalopram, and stress+escitalopram. The offspring were analyzed at adulthood to determine the long-term effects of prenatal stress and/or escitalopram exposure. Evaluation at baseline showed statistically significant changes in several endocrine measurements, but these were not believed to be physiologically relevant. Microarray analysis of the amygdala, hippocampus, and hypothalamus comparing all treatment groups revealed no significantly regulated genes. RT-PCR was used to determine more subtle gene expression differences that would not be detected with a microarray analysis (i.e., <1.5-fold changes). Discrete stress and antidepressant-

regulated targets were selected for analysis in the hippocampus. Several genes related to vascular density (VegfC, vWF) and hypothalamic-pituitary-adrenal axis function (Gr, Pomc) were altered by prenatal treatments. No changes were observed in several serotonin receptor subtypes or TrkB. These results show that alterations in the in utero environment modeled on maternal depression and its treatment may result in long-term effects on the gene expression in adult offspring. Studies are underway to investigate the importance of these observations.

PS 1640 FACIAL ANOMALIES IN A RAT MODEL WITH ETHANOL: ANIMAL FRIENDLY MARKERS FOR FETAL ALCOHOL SYNDROME (FAS).

R. Slieker, L. vd Horst, R. Nederlof, M. Otto and D. De Groot. *TNO Quality of Life, Zeist, Netherlands.* Sponsor: R. Woutersen.

Background. Fetal alcohol syndrome (FAS) is the teratogenic consequence of excessive maternal consumption of alcohol during pregnancy. In addition to neurocognitive and behavioural disabilities children with FAS show facial anomalies including short palpebral fissures, a hypoplastic philtrum and a thin upper lip. These anomalies are used for the diagnosis of FAS in children. Goal. Three questions were raised: 1. Are similar facial anomalies present in a rat model of prenatal exposure to ethanol? 2. Can facial anomalies be derived and quantified from photographs, taken straightforward from the rat's snout? 3. Can facial anomalies thus serve as animal friendly markers for normal development and development of FAS in a rat model thereby reducing or even replacing conventional daily testing of senses and reflexes of rat pups during the preweaning period, or testing of cognition and motor performance during adolescence and adulthood? Strategy. A recently developed protocol for an Extended One Generation Reproduction Toxicology Study (OECD EOGRTS) was performed in rats, exposing them daily to ethanol via drinking water (0, 1.5, 4.0, 6.5, 9.0, 11.5, 14%) from 2 weeks pre-mating to postnatal day (PND) 70 of the offspring. For the purpose of this research, the offspring was photographed with a high resolution camera on PND8, PND37, PND61 and PND70. A number of parameters was selected analogues to analysis in man, i.e. height of the philtrum, width and height of the nose and the width of the lower lips. Parameters were measured digitally with correction for the magnification used. Conventional daily testing of senses and reflexes of the rat pups, testing of cognition and motor performance at later test age was performed as well. Results / Conclusions. Facial anomalies, similar to children with FAS, were observed in ethanol exposed rats showing ethanol induced retardation in conventional guideline testing, i.e. significant reduction in height of the philtrum and changes in width / height of the nose, thus offering early and animal friendly markers for FAS in a rat model with ethanol.

PS 1641 DEVELOPMENTAL ARSENIC EXPOSURE INCREASES PERINATAL MORTALITY AND CAUSES LIVER INFLAMMATION IN FEMALE MICE.

M. W. Carmody, A. R. King, A. E. Hill and R. J. Sommer. *Biology Department, Bates College, Lewiston, ME.*

Recently, more attention is being paid to the risks of early life exposure to arsenic. In our laboratory, developmental exposure of C57BL/6 mice to 50 or 500 ppb sodium arsenite (given in maternal drinking water 1 week prior to mating until PND 21) decreased the average number of live pups per litter on PND 2 (7.1 ± 0.4 to 4.7 ± 0.9 and 4.7 ± 1.1 neonates/litter, mean ± SEM, for the 0, 50, 500 ppb groups, respectively, N=9-10). Small decreases in uterine implantation sites (8.7 ± 0.4, 7.9 ± 0.3, 7.7 ± 0.4, N=14-16) may have contributed to the smaller litter size on PND 2. However, no difference in maternal body weight gain during pregnancy, combined with no change in the percentage of the number of live fetuses per uterine implantation sites on gestation day (gd) 18, indicates that more pup lost occurred late in gestation or soon after birth. No additional pup mortality occurred after PND 2. On PND 21, all offspring were weaned to cages with "clean" water (no arsenic). Thus, arsenic exposure in our study occurred only from the time of conception until PND 21. At 4 months of age, females exposed to arsenic early in life had livers with many perivascular and lobular collections of leukocytes. Small collections of inflammatory cells were observed in control female livers but the number and size of inflammatory collections increased in females developmentally exposed to arsenic. Control females averaged 5.2 ± 1.8 small lesions/mm^2 (N=5), whereas 50 and 500 ppb females averaged 13.1 ± 3.7 (N=7) and 13.5 ± 5.6 (N=4) small to large lesions per mm^2 of liver. At 4 months of age, liver organ weight was decreased (1.12 ± 0.04, 1.19 ± 0.06, 0.93 ± 0.03 g, N=6-8) and the percentage of hepatocytes with multiple nuclei were increased (46 ± 4, 47 ± 4, 64 ± 6 %, N=6-8) in female offspring exposed to 500 ppb arsenic from conception to PND 21. In summary, developmental arsenic exposure caused a modest increase in perinatal mortality and caused liver toxicity in female mice in adulthood. Supported by NIH R15 ES019108 and Bates College.

PS 1642 MICRORNA EXPRESSION PROFILES AFTER DEVELOPMENTAL EXPOSURE OF ZEBRAFISH (*DANIO RERIO*) EMBRYOS TO TCDD.

N. Aluru[1], M. J. Jenny[1, 2] and M. E. Hahn[1]. [1]*Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA and* [2]*Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.*

MicroRNAs are an important group of small non-coding RNAs that regulate mRNA expression. MicroRNA roles in early embryonic development are well established, and their disruption during development can cause abnormalities. However, little is known about the effect of developmental toxicants on microRNA expression. We hypothesized that the developmental exposure of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters microRNA expression patterns in embryos. In an initial experiment, zebrafish embryos were exposed to 5 nM TCDD or DMSO at 30 hr post-fertilization (hpf) for 1 hr and sampled at 36 and 60 hpf, prior to and at the onset of TCDD-induced phenotypic abnormalities, respectively. MicroRNA expression profiles were determined using microarrays (Agilent and Exiqon) and qRT-PCR. Microarray results revealed modest changes in microRNA expression. Exiqon microarray revealed altered expression of 8 and 14 microRNAs at 36 and 60 hpf, respectively; Agilent microarray revealed no changes at 36 hpf and 8 microRNAs altered at 60 hpf. Both Exiqon and Agilent platforms showed similar patterns for miR-451, 23a, 23b, 24 and 27e. These microRNAs are known to target genes involved in erythrocyte maturation (miR-451) and cardiac development (miRs-23a, 23b and 24). To characterize the temporal profiles of TCDD-induced changes in microRNA expression, we exposed embryos to 5 nM TCDD or DMSO for 1 hr at 4 hpf and sampled at 24, 48, 60, 72, and 96 hpf. The greatest changes occurred later in development (72 hpf). These results suggest that TCDD exposure causes modest changes in expression of microRNAs, including some that are critical for hematopoiesis and cardiac development. Ongoing studies are evaluating effects of other teratogens on microRNA expression and are comparing deep sequencing (Illumina, SOLiD) and microarray approaches for measuring microRNA profiles. [Supported by R21ES017304]

PS 1643 MIRNAS ARE ESSENTIAL FOR BILE ACID HOMEOSTASIS DURING LIVER DEVELOPMENT.

Y. J. Cui, J. Liu, Z. Fu, Y. Guo and C. D. Klaassen. *Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

One critical pathway that is often compromised in many pediatric liver diseases is bile acid (BA) homeostasis, leading to cholestasis and malnutrition in children. Growing evidence suggests that miRNAs are implicated in a wide range of pathological processes in liver by regulating the mRNA stability and protein formation of genes, but very little is known about miRNAs in normal liver development. The purpose of the present study was to determine the roles of mRNAs in BA homeostasis during liver development. Mice with hepatocyte-specific depletion of Dicer, an essential enzyme in producing all mature miRNAs were engineered. Dicer depletion markedly increased BA concentrations from 20 to 45-days of age (UPLC-MS/MS), corresponding to profound progressive hepatocyte degeneration and compensatory cell proliferation (H&E). Surprisingly, although BA concentrations were high, the mRNA of the rate-limiting BA-synthesizing enzyme, Cyp7a1, generally did not change in livers of Dicer-null mice (RT-PCR). Conversely, there was a marked decrease in the mRNAs of Sult1b1 and Ugt2b1 (enzymes for BA-detoxification), as well as Ugt1a1 (an enzyme for bilirubin-conjugation). For BA-transporters, there was a decrease in both the mRNAs and proteins of the BA-uptake transporters Ntcp and Oatp1b2, as well as an increase in the BA-efflux transporter Mrp4 in Dicer-null livers. However, the mRNA of the rate-limiting BA-efflux transporter Bsep did not change in Dicer-null livers during development, and in fact, the Bsep protein even decreased at 20-days of age (western blot), which correlated with a decrease in both mRNAs and proteins of the major BA-sensor, FXR. In conclusion, depletion of mature miRNAs produced cholestasis in developing livers corresponding to impaired compensatory regulation of many essential BA-processing genes, highlighting the importance of miRNAs in maintaining normal BA homeostasis during liver development. (Supported by NIH grants ES-07078, ES-09649, ES-09716, ES-013714, and ES-021940.)

PS 1644 AN INHALATION DEVELOPMENTAL TOXICITY STUDY OF N-ETHYL ACETAMIDE IN RATS.

J. Domoradzki[1], T. Edwards[2] and D. Kirkpatrick[2]. [1]*Dow Corning Corporation, Auburn, MI and* [2]*WIL Research Laboratories, LLC, Ashland, OH.*

Developmental effects of alkylated acetamides have been reported in laboratory animals by various routes of administration. Since inhalation of N-ethyl acetamide (NEA) is a potential route of worker exposure, data from whole-body inhalation exposures of rats to NEA were relevant to health risk assessment. The objective was to

determine the potential of NEA, to induce developmental toxicity after maternal exposure via whole-body vapor inhalation (6 h per day) from implantation to 1 day prior to parturition, to characterize maternal toxicity, and to determine a NOAEL for maternal and developmental toxicity. Groups of bred female Crl:CD(SD) rats (25/group), ~ 14 weeks of age, were exposed to either filtered air or vapor atmospheres of NEA during gestation days (GD) 6-19. Target concentrations were 4, 10, and 40 (highest achievable) ppm. On GD 20, a laparohysterectomy was performed. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations and developmental variations. All animals survived to the scheduled necropsy on GD 20. There were no test substance-related clinical findings noted at the daily examinations, mid-point of exposure, or 1 hour following completion of exposure. Mean body weights, body weight changes, net body weights, net body weight changes, gravid uterine weights, and food consumption in the 4, 10, and 40 ppm groups were similar to that of the control group throughout the entire exposure period. Intrauterine growth and survival and external, visceral, and skeletal fetal morphology were unaffected by test substance administration at all exposure levels. No signs of maternal or developmental toxicity were observed at any exposure level evaluated. A NOAEL of 40 ppm was assigned for maternal and prenatal developmental toxicity.

PS 1645 EFFECTS OF THALIDOMIDE ON NORMAL FETAL GROWTH AND HEART RATE IN CYNOMOLGUS MONKEYS.

C. Diabo, M. Kob, C. Cohle, K. Griffin, B. Lewis, A. Kuzmin, S. Magness and A. S. Faqi. *Drug Safety Evaluation, MPI Research, Mattawan, MI.*

Due to increasing numbers of biologics, developmental toxicity testing in nonhuman primates (NHPs) has become more common as NHPs are often the only species that express pharmacologic responses similar to humans. One of the technical challenges in the developmental toxicity studies of NHPs is the establishment of pregnancy and ensuring the normal development of the fetus during the study. In nonhuman primates ultrasound is used as in humans to determine pregnancy, the embryo/fetal size, weight and to measure growth thus making sure the fetus is developing according to plan. The objective of this study was to determine whether administration of thalidomide during the window of sensitivity in pregnant NHPs will adversely impact fetal growth and heart rate.
Sexually mature Vietnamese female cynomolgus monkeys were bred in-house for the study. Prior to mating menstrual cycle of each female was monitored for at least 3 months. Pregnancy was confirmed on Gestation Days (GDs) 18-20 using ultrasound. Pregnant cynomolgus monkeys received either vehicle (n=10) or 7.5 mg/kg/day of thalidomide (n=10) on GDs 26, 27 and 29. C-section was performed on 50% of the animals on GD 100. The remaining 50% of the animals (n= 5/group) were allowed to deliver naturally. During pregnancy ultrasound measurements including fetal crown-rump length (GD 48-52), cranial, and femoral measurements and heart rate were performed. Typical thalidomide-related malformations were noted in the fetuses of the thalidomide group. No statistical differences in fetal growth as determined by fetal crown-rump length, cranial and femur length measurements were observed between control animals and the thalidomide group. Likewise, analysis of fetal heart rates didn't indicate significant difference among the groups (control: 372.2 ± 15.0 vs. thalidomide: 379.5 ± 13.0 BPM). Based on the results of this study, there seems to be no indication that thalidomide induces effects on fetal growth and heart rate in pregnant monkeys when exposed during the window of sensitivity.

PS 1646 COMPARATIVE EVALUATION OF LEARNING AND MEMORY IN RATS USING MORRIS MAZE VIDEO TRACKING SYSTEM AND PASSIVE AVOIDANCE.

C. Lanphear, A. Terpstra, A. Rohr and A. S. Faqi. *Drug Safety Evaluation, MPI Research, Mattawan, MI.*

The objective of the study was to determine the difference in sensitivity between Morris Maze and Gemini systems of detecting effects on learning and memory impairment when rats were exposed to Scopolamine. Four groups of 25 rats/sex/group were administered subcutaneously either the vehicle (0.9% Sodium Chloride for Injection) [Groups 1 and 3] or Scopolamine (Groups 2 and 4) on Days 1-5, at a dose volume of 1 mL/kg. Daily (Days 1-5), each rat was administered either the vehicle or Scopolamine 20 minutes prior to the beginning of the testing with the Morris maze. The procedure consisted of placing each rat in a large circular pool of water and the rat was expected to escape from water onto a hidden platform whose location can be identified only using spatial memory. A total of 5 trials was performed with the fifth trial (Day 5) described as probe trial conducted with the platform removed. The time to reach the platform, path length, cumulative path length, and swim speed were measured for each interval. For passive avoidance, trials to criterion with stop were performed. This involves 5 sessions where the animal received foot shock until they reached a criterion, defined as remaining passive for 2 consecutive 180 s trials. Significant increase in the time to reach the platform (latency), path length, cumulative path length and a decrease in swim speed for males and females treated with Scopolamine were observed. Review of the pictograms on Day 5 showed that Scopolamine treated animals have not learned the location of the hidden platform after five consecutive testing days. In passive avoidance, the number of Scopolamine treated males that did not pass increased significant (32% vs. 4% of controls). Scopolamine treated females were comparable with the vehicle controls. Based on the results of this study Morris water-maze is a more sensitive test than the step-through passive avoidance procedure when evaluating spatial learning and memory.

PS 1647 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) INHIBITS EPICARDIAL DEVELOPMENT IN THE EMBRYONIC ZEBRAFISH HEART.

J. S. Plavicki, W. Heideman and R. E. Peterson. *School of Pharmacy, University of Wisconsin at Madison, Madison, WI.*

Exposure to 2, 3, 7,8-tetrachlorodibenzo-p-dioxin (TCDD) during early embryonic development produces numerous cardiac phenotypes in zebrafish, including decreased numbers of cardiomyocytes, heart looping defects, valve defects and reduced cardiac output. The window of TCDD sensitivity during embryogenesis correlates with the formation of the outermost layer of the heart, the epicardium, which plays critical roles in cardiac development. The epicardium originates from the proepicardial organ (PEO) and contains cardiac progenitor cells that contribute to both the myocardium and the coronary vasculature. In addition, the epicardium is necessary for maturation of the cardiac conduction system. We hypothesized that disruption of epicardial development may account for the cardiac toxicity produced by TCCD exposure. To test whether TCDD disrupts embryonic epicardial development, we examined the effects of TCCD exposure (1.0 ng/ml waterborne) on epicardium formation by examining sections stained with hematoxylin and eosin (H&E). We also followed the expression of known epicardial markers across multiple stages of embryonic development with in situ hybridization, fluorescence immunocytochemistry (F-ICC) and an epicardial GFP reporter using confocal microscopy. Strikingly, TCCD exposure during the first 24 hours post fertilization (hpf) caused a complete the loss of the embryonic epicardium. The expression of the epicardial markers including Wilms' Tumor 1 (wt1) and tcf21/epicardin were decreased in TCDD exposed embryos as compared to vehicle controls at 48, 72, 96 and 120 hpf. The H&E stained sections confirmed that epicardial cells were absent in TCCD exposed embryos. Time course and other studies were used to determine whether TCDD alters PEO formation or acts after the specification of epicardial cell fate. Our results show that brief exposure to TCDD in the parts per trillion to parts per billion levels can prevent the formation of an essential component layer of the developing vertebrate heart. Supported by NIH Grant ES012716 and UW Sea Grant.

PS 1648 MINOCYCLINE AND DOXYCYLINE, BUT NOT TETRACYCLINE, DECREASE LIVER AND KIDNEY INJURY AFTER HEMORRHAGIC SHOCK AND RESUSCITATION IN MICE.

A. Kholmukhamedov[1], C. Czerny[1], J. Hu[1], J. Schwartz[1] and J. J. Lemasters[1, 2]. [1]*Pharmaceutical & Biomedical Sciences, Medical University of South Carolina, Charleston, SC and* [2]*Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC.*

Background: Recent evidence suggests that despite recovery of hemodynamics by fluid resuscitation, liver and kidneys experience progressive injury. Minocycline and doxycycline are tetracycline derivatives that are protective to various organs in models of hypoxic, ischemic and oxidative stress and which may act by preserving mitochondrial function. Our overall Aim was to determine whether minocycline and doxycycline protect liver and kidney in a mouse model of HS/R. Methods: Under pentobarbital anesthesia, C57BL6 mice were hemorrhaged to 30 mm Hg for 3 h and then resuscitated with shed blood plus half the volume of lactated Ringer's solution containing tetracycline, minocycline, doxycycline or vehicle. For the pretreatment studies, drugs were administered via intraperitoneal injection and then immediately after the blood resuscitation. Serum alanine aminotransferase (ALT) and creatinine, liver and kidney histology were assessed 6 h after resuscitation. Results: ALT increased to 1988 U/L after resuscitation with vehicle and to 1963

U/L after tetracycline. Minocycline and doxycycline post-treatment decreased ALT to 857 and 789 U/L, respectively ($p<0.001$). Minocycline and doxycyline pre-treatment also decreased ALT to 776 and 660 U/L ($p<0.001$). After resuscitation with vehicle, blood creatinine increased to 273 μM. Minocycline and doxycycline post-treatment decreased blood creatinine to 109 and 92 μM, respectively ($p<0.05$). Minocycline and doxycycline pre-treatment also decreased creatinine to 76 and 83 μM ($p<0.05$). Conclusion: Minocycline and doxycycline are as efficient when given after the blood resuscitation as they are given before the hemorrhagic shock for the alleviating liver and kidney injury after HS/R, whereas tetracycline does not show any protective effect. Minocycline and doxycycline are safe FDA-approved drugs that might therefore be used to mitigate liver and kidney injury after HS/R.

PS 1649 DOXYCYCLINE AND MINOCYCLINE, BUT NOT OTHER TETRACYCLINE DERIVATIVES, INHIBIT THE MITOCHONDRIAL PERMEABILITY TRANSITION AND MITOCHONDRIAL IRON UPTAKE.

J. Schwartz[1], E. Holmuhamedov[1] and J. J. Lemasters[1, 2]. [1]*Pharmaceutical & Biomedical Sciences, Medical University of South Carolina, Charleston, SC and* [2]*Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC.*

BACKGROUND: Minocycline and doxycycline are unique among tetracycline derivatives in their ability to inhibit mitochondrial Ca^{2+} uptake and protect hepatocytes and other cells from chemical hypoxia and ischemia/reperfusion (I/R) injury. The AIM of this study was to evaluate the ability of various tetracycline-derived compounds to block mitochondrial iron uptake and the pathophysiological mitochondrial permeability transition (MPT). **METHODS:** Isolated rat liver mitochondria (0.5 mg/ml) were exposed to vehicle (control group), 100 nM Ru360 (inhibitor of the mitochondrial electrogenic calcium uniporter), 2 μM cyclosporin A (CsA, an MPT inhibitor), various tetracycline derivatives, and lastly 250 μM $FeSO_4$ or 250 μM $CaCl_2$. Mitochondria exposed to $CaCl_2$ were monitored for swelling, an indicator of the MPT, detected as decreased absorbance at 540 nm. $FeSO_4$-stimulated mitochondrial respiration, a measure of electrogenic Fe^{2+} uptake, was measured with a Clark oxygen electrode. **RESULTS:** Minocycline, doxycycline, Ru360 and CsA each inhibited Ca^{2+}-induced mitochondrial swelling. Doxycycline inhibited swelling at lower concentrations than minocycline. Minocycline, doxycycline, and Ru360 also inhibited Fe^{2+}-induced respiratory stimulation by 67%, 72% and 100% respectively. Other tetracycline derivatives, including tetracycline, did not block MPT induction or inhibit Fe^{2+}-stimulated respiration. **CONCLUSION:** Minocycline, doxycycline, and Ru360 are all inhibitors of mitochondrial Ca^{2+} and Fe^{2+} uptake, but non-cytoprotective tetracycline-derived compounds are not. Ca^{2+} and Fe^{2+} uptake is via the electrogenic calcium uniporter, the only known transporter of both Ca^{2+} and Fe^{2+} into mitochondria. Thus, inhibition of Fe^{2+} uptake, Ca^{2+} uptake or both may underlie cytoprotection by Ru360, minocycline and doxycycline.

PS 1650 LYSOSOMES, COMPOUND ACCUMULATION, AND *IN VITRO* TOXICITY.

S. Lu[1], S. Nadanaciva[2], R. Swiss[2], B. Jessen[1] and Y. Will[2]. [1]*Drug Safety Research and Development, Pfizer Inc., San Diego, CA and* [2]*Compound Safety Prediction, Pfizer Inc., Groton, CT.*

Lysosomes are membrane-enclosed compartments filled with acid hydrolytic enzymes that digest macromolecules from the endocytic, autophagic, and phagocytic membrane-trafficking pathways. Weakly basic lipophilic compounds can accumulate in lysosomes, a process known as lysosomotropism. This can cause lysosomal labilization, potentially leading to sublethal damgage, and even apoptosis and necrosis. The relationship between lysosomal accumulation, impairment of cellular function, cellular damage and eventual manifestations of target organ toxicity has not been fully elucidated. Here we used four different data sets to investigate how physiochemical properties of compounds that accumulate in lysosomes may correlate with various in vitro toxicity end points. In the first data set, 1500 compounds were tested in the HepG2 ATP depletion assay and the IC50 values were analyzed relative to the physiochemical properties. The majority of non-lipophilic compounds were not toxic up to 300μM and the percentage of toxic compounds was substantially higher for lipophilic compounds with a fairly high basic pKa. In the second data set, 360 hepatotoxicants were tested for 8 different mechanistic endpoints using high content analysis. Lysosomal mass was found to be the most sensitive parameter measured and this correlated well with the compounds' physiochemical properties. In a third experiment, 200 cardiotoxicants were evaluated in an eight-parameter high content analysis using H9c2 cells. Lipid accumulation was the most sensitive parameter and compounds flagging for this endpoint were predominantly basic and lipophilic. The 200 cardiotoxicants were further validated by a subsequent assay using LysoTracker dye. The data showed that compounds which accumulated in lysosomes were basic and lipophilic. Overall, this data suggested that compound accumulation in lysosomes could be a mechanism contributing to in vitro toxicity. Further work will need to evaluate the contribution of lysosomotropism to in vivo organ toxicity.

PS 1651 LYSOSOMAL IRON RELEASE AND MITOCHONDRIAL IRON UPTAKE CONTRIBUTE TO ISCHEMIA-REPERFUSION INJURY TO RAT HEPATOCYTES.

X. Zhang and J. J. Lemasters. *Medical University of South Carolina, Charleston, SC.*

Reactive oxygen species (ROS)-mediated onset of mitochondrial permeability transition plays an important role in injury of liver ischemia-reperfusion (IR). Iron is a critical catalyst for ROS formation, and intracellular iron levels are promoted in ischemia of brain and heart. Our aim was to determine the role of intracellular chelatable iron in IR-induced cell injury in rat hepatocytes. Cultured hepatocytes were incubated anoxically at pH 6.2 for 4 h and reoxygenated at pH 7.4 to simulate IR. Mitochondrial membrane potential and chelatable ferrous iron were monitored by confocal microscopy of tetramethylrhodamine methyl ester and quenching of ester-loaded calcein fluorescence. Cell killing was assessed by propidium iodide fluorometry. Cytosolic calcein fluorescence began to quench within 1 h, and near complete quenching was evident at the end of ischemia. Calcein quenching was suppressed by pretreatment with the iron chelators desferal (1 mM) and starch-desferal (s-desferal, 1 mM) 1 h before ischemia. Prevention of quenching of cytosolic calcein by s-desferal indicated that chelatable iron was being released by lysosomes. Ischemia also induced quenching of mitochondrially loaded calcein and dequenching of lysosomal calcein fluorescence. Mitochondrial calcein quenching was prevented by Ru360 (20 μM), an inhibitor of mitochondrial calcium uniporter, indicating that chelatable iron released from lysosomes was being taken up by mitochondria during ischemia. Importantly, mitochondrial depolarization and cell killing after reperfusion were reduced by pretreatment of desferal, s-desferal and Ru360 prior to ischemia. These results indicate the release of chelatable iron from lysosomes and uptake by mitochondria occurs during ischemia, which promotes depolarization of mitochondria and cell killing after reperfusion. Chelation of lysosomal iron or blockade of mitochondrial iron uptake attenuates IR-induced mitochondrial dysfunction and cell death. Taken together, the data indicate that lysosomal iron release and mitochondrial iron uptake contribute to IR injury to rat hepatocytes.

PS 1652 THE CYTOTOXIC INTERACTION BETWEEN HYPOXIA AND NEUTROPHIL ELASTASE IN A MURINE HEPATOMA CELL LINE IS INDEPENDENT OF HIF-1α SIGNALING.

E. M. Sparkenbaugh, M. A. Nothem, P. E. Ganey and R. A. Roth. *Pharmacology and Toxicology, Michigan State University, Lansing, MI.*

The liver is sensitive to pathological conditions such as toxicoses, sepsis, and ischemia/reperfusion, which are associated with tissue hypoxia (HX) and the presence of activated neutrophils that secrete the serine protease elastase (EL). We demonstrated previously that cotreatment of Hepa1c1c7 cells with nontoxic concentrations of HX and EL caused synergistic cell death that is dependent on p38 signaling. Cells respond to HX through hypoxia-inducible factor-1α (HIF-1α), a transcription factor that directs the transcription of many genes, including the cell death gene BNIP3. We tested the hypothesis that HX/EL cytotoxicity is dependent on HIF-1α signaling. Hepa1c1c7 cells were treated with 0 or 15 U/mL EL in an oxygen-replete (100 mmHg) or hypoxic (10 mmHg) environment for 0-24 hours. Exposure to HX for 1 hour caused an increase in nuclear HIF-1α protein that was further increased by HX/EL, suggesting that HX/EL cotreatment enhances HIF-1α signaling in Hepa1c1c7 cells. HX caused a significant increase in the BNIP3 mRNA:HPRT ratio from 0.5 ± 0.2 to 2.9 ± 0.3, and cotreatment with EL enhanced this significantly to 7.7 ± 0.8. Pretreatment with a p38 inhibitor reduced the BNIP3:HPRT ratio to 4.9 ± 1.7 in HX/EL-cotreated cells. There was a corresponding increase in BNIP3 protein in HX/EL-cotreated cells compared to HX or EL alone at 10 and 12h. To evaluate the role of HIF-1α signaling in the interaction between HX and EL, cells were treated with $CoCl_2$ (200 μM), deferoxamine mesylate (DFX, 200 μM) or dimethyloxallyl glycine (DMOG, 500 μM) to activate HIF-1α independently from HX. Neither DFX nor DMOG were cytotoxic to

Hepa1c1c7, nor did they enhance the cytotoxicity caused by EL. $CoCl_2$ was cytotoxic by itself, but reduced EL cytotoxicity. Results suggest that HIF-1α activation and signaling is increased in HX/EL-cotreated cells, but the cytotoxic interaction between HX and EL acts through other signaling pathways. (Supported by R01 ES004139 and T32 ES007255.)

PS 1653 THE ROLE OF MITOCHONDRIA IN AMIODARONE-INDUCED APOPTOSIS IN RAT PLEURAL MESOTHELIAL CELLS.

E. S. Taneva and J. M. Cerreta. *PHS, St. Johns University, Queens, NY.* Sponsor: L. Trombetta.

Amiodarone is a class III antiarrhythmic agent whose clinical utility may be limited due to the incidences of amiodarone-induced pulmonary toxicity (AIPT). The course of AIPT includes alveolitis, phospholipidosis, and irreversible fibrosis. A number of studies have indicated a key role of epithelial cell apoptosis in the pathogenesis of AIPT. The purpose of this study was to investigate the cytotoxic effect of amiodarone (AM) and to elucidate the molecular pathway involved in the AM-induced apoptotic machinery in rat pleural mesothelial cells (RPMCs). RPMCs were cultured in Ham's F-12 medium containing 15 % fetal bovine serum at 37 °C in a humidified 5 % CO2 environment. The cytotoxicity of AM was tested by using the methylthiazol diaphenyltetrazolium bromide (MTT) method. Confluent cultures were exposed to increasing concentrations of the drug (12. 5, 25, 50, and 100 μg/ml) diluted in culture medium over a 24 hour period. All AM concentrations except for the lowest AM concentration (12.5 μg/ml) significantly decreased the cell viability. The cytotoxicity was concentration-dependent and the calculated IC50 value was 50 μg/ml. Additional cultures were pre-treated for 2 hours with the pan-caspase inhibitor Z-VAD-fmk followed by co-treatment with the IC50 value of AM and varying concentrations of the inhibitor (1, 5, and 10 μg/ml). Z-VAD-fmk demonstrated a protective effect on the cell viability at all tested concentrations. The cells were stained after AM treatment with the cationic fluorophore Rh123 (1 μg/ml for 30 min at 37 °C) to ascertain AM initiates the mitochondria pathway of the programmed death cascade. AM leads to a decrease in the membrane potential-driven accumulation of Rh123 in the inner mitochondrial membrane within 2 hours after treatment with the drug. These data indicate that AM induces cell death in RPMCs via the mitochondrial apoptotic pathway. Such results may have implications in understanding the molecular mechanism of AM-induced lung toxicity.

PS 1654 ACUTE SINGLE-MITOCHONDRION RESPONSES TO 1, 3-DINITROBENZENE IN ASTROCYTES.

L. Maurer[1], I. C. Speirs[1], J. Fernandez[1], D. Song[2], T. Epstein[3], A. Jureziz[1], S. Steiner[1] and M. A. Philbert[1]. *[1]Department of Environmental Health Science, Toxicology, University of Michigan, Ann Arbor, MI, [2]Mechanical Engineering Department, University of Michigan, Ann Arbor, MI and [3]Chemistry Department, University of Michigan, Ann Arbor, MI.*

As cells in culture age they manifest biochemical changes resulting in loss of function or cell death. While endpoints such as cell viability are useful, they may not capture subtle changes that auger changes in cell function. 1,3-Dinitrobenzene (DNB) is cytotoxic to rat brainstem astrocytes in vivo. Mitochondria in cultured immortalized astrocytes (DI-TNC1) exposed to DNB exhibit intermittent loss of inner mitochondrial membrane potential (ΔΨmt) with repolarization prior to permanent depolarization. Dynamics in DI-TNC1 cells were measured in response to DNB exposure to investigate early sequelae as a function of cellular 'age'. To observe flickering events, DI-TNC1 cells were exposed to DNB (0–600 μM in 0.1% DMSO) and ΔΨmt was monitored discontinuously using tetramethylrhodamine methyl ester (100 nM). Cells were maintained in an environmental chamber for up to 15 minutes and imaged using an Olympus IX81. Original micrographs were converted to binary images in MatLab. Mean mitochondrial aspect ratios were calculated and modeled as a function of DNB concentration, exposure time, and cell passage (cellular age). Cells exposed to DNB exhibited a negative, age-dependent relationship between concentration and time to initial flickering events. These events were statistically significant ($p<0.05$) when stratified by age; older cells displayed initial flickering of ΔΨmt earlier than younger cells. Changes in mean mitochondrial aspect ratio were statistically significant as a function of DNB concentration and exposure time when stratified by cellular age ($p<0.05$). These findings suggest that as cells age, they become more sensitive to mitochondrial poisons such as DNB. Increased sensitivity can be monitored by observing subtle initial depolarization/repolarization and morphological events in individual mitochondria. Supported by NIH 2RO1 ES008846 (LM, ICS, JF, DHS, AJ, MAP) and NIH 2T32 ES007062 (LM).

PS 1655 ARE REACTIVE OXYGEN SPECIES REQUIRED FOR AUTOPHAGY?

J. Jiang[1], D. A. Stoyanovsky[1], A. Maeda[1], J. S. Greenberger[2] and V. E. Kagan[1]. *[1]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and [2]Radiation Oncology, University of Pittsburgh, Pittsburgh, PA.*

Reactive oxygen species (ROS) have been suggested to play an essential role in the autophagy signaling pathway. No direct proof, however, has been obtained largely due to interaction of apoptosis and autophagy. We dissected the autophagy and apoptosis pathways by knocking down the expression of cytochrome c, the key component of mitochondria-dependent apoptosis pathway, in HeLa cells using shRNA procedure. The mitochondrial electron transport chain was compromised due to the lack of the electron shuttle between mitochondrial respiratory complexes III and IV in cytochrome c deficient HeLa1.2 cells. Importantly, ROS generation could be manipulated without affecting apoptosis. Staurosporine (STS, non-oxidant) induced a rapid and robust LC3-I/II conversion and mitochondrial degradation in HeLa1.2 cells in the absence of apoptosis as evidenced by western blot and transmission electron microscopy. No superoxide and hydrogen peroxide generation, however, were detected in STS-treated HeLa1.2 cells using dihydroethidium and Amplex Red assay. In addition, a membrane permeable antioxidant PEG-SOD with catalase exerted no effect on STS-induced LC3-I/II conversion and mitochondrial degradation. Furthermore, autophagy was readily detectable in STS-treated mitochondrial DNA deficient ρ0 HeLa1.2 cells, in which the electron transport and the ability of ROS generation were completely disrupted. In summary, our data indicate that ROS are not essential signals for the autophagy induction. Supported by NIAID U19-AI068021, U19-A8068021, HL70755, and HL094488.

PS 1656 CONNEXIN32 HEMICHANNELS FACILITATE THE APOPTOTIC-TO-NECROTIC TRANSITION DURING FAS-MEDIATED HEPATOCYTE CELL DEATH.

M. Vinken[1], E. Decrock[2], E. De Vuyst[2], M. De Bock[2], V. Roosmarijn[3], B. De Geest[4], J. Demeester[4], N. Sanders[5], T. Vanhaecke[1], L. Leybaert[2] and V. Rogiers[1]. *[1]Toxicology, Free University Brussels, Brussels, Belgium, [2]Basic Medical Sciences-Physiology Group, Ghent University, Ghent, Belgium, [3]Molecular Biomedical Research, Ghent University, Ghent, Belgium, [4]General Biochemistry and Physical Pharmacy, Ghent University, Ghent, Belgium and [5]Gene Therapy, Ghent University, Ghent, Belgium.*

BACKGROUND/AIMS: Although it is widely accepted that the establishment of gap junctional intercellular communication (GJIC) is a prerequisite for maintaining liver homeostasis, its role in the occurrence of apoptotic hepatocyte cell death still remains elusive. Most hepatic gap junctions are formed by two hemichannels of neighboring hepatocytes, which are composed of connexin32 (Cx32). The present study was set up to investigate the fate of Cx32 and its channels in Fas-mediated hepatocellular apoptosis. METHODS: Primary hepatocytes were exposed to Fas ligand, and Cx32 expression was studied by means of immunoblotting and qRT-PCR analysis. GJIC was monitored by fluorescence recovery after photobleaching analysis. Cx32 hemichannels were approached through Cx32 siRNA-mediated gene silencing, cell surface biotinylation, application of a Cx32 mimetic peptide and measurement of ATP release. RESULTS: GJIC rapidly declined upon progression of the cell death response, which was associated with a decay of the gap junctional Cx32 protein pool. Simultaneously, levels of newly synthesized Cx32 protein increased and gathered in a hemichannel configuration. This particularly became evident towards the final stages of the cell death process and was not reflected at the transcriptional level. Both the silencing of Cx32 expression and the inhibition of Cx32 hemichannel activity prior to cell death induction resulted in a delayed termination of the cell death response. CONCLUSIONS: (i) Cx32 hemichannels facilitate the apoptotic-to-necrotic transition, which typically occurs in the final stage of Fas-mediated hepatocyte cell death. (ii) Primary hepatocyte cultures are suitable in vitro tools to elucidate the mechanisms that underlie hepatocyte cell death.

PS 1657 DECIPHERING THE ROLE OF FASL IN REGULATING C-FLIP EXPRESSION IN GERM CELLS.

Y. Lin, P. Yao and J. H. Richburg. *Center for Molecular and Cellular Toxicology, College of Pharmacy, Division of Pharmacology and Toxicology, The University of Texas at Austin, Austin, TX.*

FasL (TNFSF6) is a ligand belonging to the tumor necrosis factor (TNF) superfamily of proteins. This ligand is expressed by testicular Sertoli cells and is implicated in the control of germ cell apoptosis. Our recent findings revealed that young

peri-pubertal FasL-/- mice show a dramatic increase in the basal germ cell apoptotic rate (20.58%) as compared to the C57BL/6J wild-type strain (5.16%). We have previously shown that exposure of young C57BL/6J mice to the Sertoli cell toxicant mono-(2-ethylhexyl) phthalate (MEHP) causes a FasL/Fas-dependent increase in the germ cell apoptotic rate. Surprisingly, exposure of young FasL-/- mice to MEHP caused a decrease in the germ cell apoptotic rate (~10% after 12 h of exposure). The expression of the c-FLIP protein, a well-described negative regulator of death receptor activation, was rapidly and strongly induced after MEHP exposure in peri-pubertal FasL-/- mice by western blot, suggesting that a lack of FasL expression may change the sensitivity of germ cells to toxicant-induced damage. Immunohistochemical analysis of testicular cross sections revealed that c-FLIP was expressed in the cytoplasm of spermatocytes and spermatids, and that MEHP-treated FasL-/- mice had a higher level of c-FLIP expression in the testis than the non-treated mice. RT-PCR analysis indicated that cflip mRNA levels were also strongly increased in MEHP-treated FasL-/- mice testes. Therefore, the regulation of c-FLIP at both transcriptional and post-translational levels seems to be a critical step in controlling germ cell apoptosis in response to certain toxicants. These observations have lead to the hypothesis that FasL is important for modulating germ cell c-FLIP expression in order to respond to the changes in Sertoli cell supportive capacity.

PS 1658 APOPTOSIS AND CELL PROLIFERATION IN HEPG2 CELLS TREATED WITH TROVAFLOXACIN AND TUMOR NECROSIS FACTOR.

K. M. Beggs, P. E. Ganey and R. A. Roth. *Pharmacology and Toxicology, Michigan State University, East Lansing, MI.*

Trovafloxacin (TVX), a fluoroquinolone antibiotic associated with idiosyncratic hepatotoxicity in humans, was shown previously to synergize with the cytokine tumor necrosis factor-alpha (TNF) to cause liver injury in mice and cytotoxicity in the HepG2 human hepatoma cell line. Apoptosis was evident histologically in vivo, and cytotoxicity in vitro depended on caspases 8 and 9. In this study we tested the hypothesis that TVX sensitizes HepG2 cells to TNF-induced apoptosis. HepG2 cells were treated with 20μM TVX, 4ng/mL TNF, or both and evaluated for apoptosis using the TUNEL method 24 and 48 hours after treatment. At both times cells co-treated with TVX and TNF displayed more TUNEL-positive cells than either treatment alone, suggesting that co-treated cells experienced DNA damage and apoptosis. In addition to activating caspases TNF promotes transcription of cell survival genes in hepatocytes. One hypothesis to explain the increased apoptotic response to TNF in the presence of TVX is that TVX inhibits transcription. To begin to address this, cells were treated with actinomycin D or cyclohexamide in the presence of TNF to evaluate whether or not these inhibitors of transcription or translation, respectively, rendered cells susceptible to TNF. Each of these agents sensitized HepG2 cells to TNF-induced cytotoxicity. In addition, TVX reduced cell proliferation at 24 and 48 hours. These data demonstrate that TVX sensitizes HepG2 cells to TNF-induced DNA damage and apoptosis. This effect might inhibit transcription of cell survival genes and decrease cell proliferation. (Supported by NIH grant R01DK061315.)

PS 1659 CAFFEINE PROMOTES UVB-INDUCED APOPTOSIS THROUGH INHIBITING THE AKT/COX-2 PATHWAYS.

W. Han, M. Ming and Y. He. *Medicine/Dermatology, University of Chicago, Chicago, IL.*

UVB in sunlight or tanning beds is a major environmental factor causing skin cancer, the most common cancer in the US. In response to UVB radiation, epidermal keratinocytes undergo apoptosis to eliminate damaged cells, thereby preventing tumorigenic transformation. Caffeine, the most widely consumed psychoactive substance, produces complex biological actions. In both humans and mice caffeine has been shown to be chemopreventive in non-melanoma skin cancer. Caffeine is known to inhibit ATR/Chk1 pathways; apoptosis increased by caffeine is found to be mediated through a p53-independent mechanism. However, the molecular basis remains unclear. Here, we have investigated how caffeine promotes UVB-induced apoptosis, using human HaCaT keratinocytes, an immortalized p53-defective cell line. Pretreatment of HaCaT cells with caffeine increased UVB-induced apoptosis and blocked UVB-induced Chk1 phosphorylation, consistent with previous findings in mice and in primary human keratinocytes. However, caffeine also significantly inhibited UVB-induced AKT phosphorylation and COX-2 up-regulation, two essential events for keratinocyte survival and tumorigenesis following UVB irradiation. In contrast, neither EGFR nor ERK signaling was affected. Blocking ATR and/or ATM pathway using siRNA targeting ATR, ATM, or both had little effect, indicating that the inhibitory effect of caffeine on AKT and COX-2 is independent of ATR or ATM. Expression of constitutively active AKT that cannot be inhibited by caffeine protected cells from apoptosis, indicating that AKT is the key target of caffeine. Blocking AKT by caffeine mediated COX-2 suppression. Disruption of either AKT or COX-2 by biochemical inhibitors or siRNA knockdown increased UVB-induced apoptosis. Taken together, these findings demonstrated that ATR-independent inhibition of AKT/COX-2 is a critical molecular mechanism by which caffeine promotes UVB-induced p53-independent apoptosis.

PS 1660 NEUROTOXIC SIGNALING PATHWAY OF BISPHENOL AF IN HIPPOCAMPAL NEURONAL CELLS: ROLE OF CALCIUM-INDUCED ROS, MAPKS, AND NF-κB.

S. Kim[2] and S. Lee[1]. [1]*Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu, Republic of Korea and* [2]*Toxicology Branch, National Toxicology Program, Morrisville, NC.*

Bisphenol AF (BPAF), a newly introduced chemical structurally related to Bisphenol A, is used extensively in fluoroelastomers, polyamides, polyesters, polycarbonate copolymers, and other specialty polymers. BPAF has been known to induce estrogen-dependent responses. However, the toxicity of BPAF is largely unknown except for endocrine-related biological effects. We investigated the neurotoxicity of BPAF and underling mechanism of actions using a mouse hippocampal cell line, HT-22 cells. BPAF significantly lowered cell viability at concentration over 100 μM in HT-22 cells. We found that BPAF induced apoptotic cell death as indicated by co-staining with Annexin V/Propidium iodide, and activation of caspase 3. To clarify the underlying mechanisms of BPAF-induced apoptosis, various signaling molecules were evaluated. BPAF increased the level of intracellular calcium, followed by the generation of reactive oxygen species (ROS). BPAF regulated the phosphorylation of mitogen-activated protein kinase (MAPK): extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), and nuclear translocation of nuclear factor (NF)-κB. Using specific inhibitors for calcium, ROS, MAPKs, NF-κB, and caspase 3, we confirmed that calcium, ROS, p38 and JNK mediated apoptotic cell death by the activation of caspase 3. Our results suggest that calcium, ROS, p38 and JNK are involved in BPAF-induced apoptotic cell death in HT-22 cells. In contrast, an NF-κB cascade and ERK were activated for survival signaling after BPAF treatment.

PS 1661 QUANTITATIVE ASSESSMENT OF [18F]-DFNSH UPTAKE BY MICROPET IMAGING AS A BIOMARKER OF ANESTHETIC-INDUCED NEURONAL DEATH.

X. Zhang[1], C. Wang[1], G. D. Newport[1], M. G. Paule[1], F. Liu[1], M. S. Berridge[2], S. M. Apana[2], G. Kabalka[3] and W. Slikker[1]. [1]*NCTR/FDA, Jefferson, AR,* [2]*3D Imaging, Little Rock, AR and* [3]*The University of Tennessee, Knoxville, TN.*

The anesthetic nitrous oxide (N2O)and isoflurane (ISO) are widely used in surgical procedures for human infants. Reports indicate that combined administration of ISO and N2O triggers neuronal apoptosis in postnatal day (PND) 7 rats. Compared with [18F]-labeled annexin V, intracellular uptake of the dansylhydrazone of p-fluorobenzaldehyde (DFNSH) may lead to improved target to background contrast ratios. Because high-resolution positron emission tomography (microPET) can provide in vivo molecular imaging at sufficient resolution to resolve neuronal activities in the rat brain, it has been proposed as a minimally invasive method for detecting apoptosis in the brain. In this study, the effect of ISO and N2O on the uptake and retention of [18F]-DFNSH in the brains of different aged rats were investigated using microPET imaging. On PND7, rat pups in the experimental group were exposed to a mixture of 70% N2O/30% oxygen and 1% ISO for 8 hours and control rat pups were exposed to room air. On PNDs 14, 21, 28, [18F]-DFNSH (18.5 MBq) was injected i.p. and thirty minutes later microPET images were obtained over 90 minutes. Radiolabeled tracer accumulation in a region of interest (ROI) in the frontal cortex was converted into Standard Uptake Values. In PND 14 rats the uptake of [18F]-DFNSH was significantly increased in the ROI in gaseous anesthetic-treated rats and the duration of tracer wash-out was prolonged. No significant difference was found in radiotracer uptake in the frontal cortex of the brains of PND 21 and 28 rats compared with same aged controls. This result, which is consistent with our previous TUNEL studies, demonstrates that enhanced apoptotic effects are apparent during early developmental stages (PND 7-14) and that no significant neuronal apoptosis occurs on or after PND 21. It is thus possible to use microPET imaging to distinguish differences in retention of [18F]–DFNSH as a minimally invasive biomarker of neuronal apoptosis in the rat brain.

PS 1662 INDUCTION OF APOPTOSIS-ASSOCIATED RIBOSOMAL RNA (RRNA) CLEAVAGE BY THE TRICHOTHECENE DEOXYNIVALENOL.

K. He[1, 2], H. Zhou[3] and J. Pestka[1, 2, 3]. [1]*Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI,* [2]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI and* [3]*Food Science & Human Nutrition, Michigan State University, East Lansing, MI.*

The trichothecene deoxynivalenol (DON) can bind to the ribosome, inhibit protein translation and induce rRNA cleavage. However, the signaling pathway leading to rRNA cleavage is still unknown. Our laboratory previously found that DON activates of mitogen-activated protein kinases (MAPKs) which mediate both gene expression and apoptosis and that both double-stranded RNA activated kinase (PKR) and hematopoietic cell kinase (Hck) are critical upstream kinases for these effects. Selective inhibitors were used to elucidate the mechanism of DON-induced rRNA cleavage in the RAW 264.7 macrophage model. RAW cells (5×106/10cm dish) were cultured overnight and pre-treated with inhibitors 1 h followed by incubation with DON (1000 ng/ml) for 6 h. Both the PKR inhibitor C-16 (>0.1μM) and Hck inhibitor PP1 (>25μM) inhibited rRNA cleavage. When inhibitors for p38, JNK and ERK were tested, only the p38 inhibitor (>5 μM) impaired rRNA cleavage. In addition, the general caspase inhibitor Z-VAD-FMK (>100uM) suppressed rRNA cleavage suggesting that this effect is apoptosis-associated. Pifithrin-α which can reversibly inhibit p53-dependent transactivation of p53-responsive genes and apoptosis, inhibited rRNA cleavage at 100 μM. Similarly, pifithrin-μ, which blocks p53 interaction with Bcl-2 family proteins and selectively inhibits p53 translocation to mitochondria completely, suppressed rRNA cleavage at 25μM. Thus DON-induced rRNA cleavage appears to be closely linked to the intrinsic apoptotic pathway sequentially involving PKR-Hck-p38-p53-caspase activation.

PS 1663 DEVELOPMENT OF NOVEL INDOLEQUINONE ANTI-TUMOR AGENTS: MECHANISM OF TOXICITY IN HUMAN PANCREATIC CANCER.

C. Yan[1], D. Siegel[1], M. Colucci[2], A. Chilloux[2], J. Newsome[2], C. J. Moody[2] and D. Ross[1]. [1]*Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO and* [2]*Chemistry, University of Nottingham, Nottingham, United Kingdom.*

A series of novel indolequinones was developed as potent antitumor agents against human pancreatic cancer. Three general classes of indolequinones with varying substitutions at the indole 2- position exhibited marked growth inhibitory activity against human pancreatic cancer both in-vitro (MTT and clonogenic assays) and in-vivo (mouse xenograft models). The pancreatic cancer cell lines PANC-1, MIA PaCa-2, and BxPC-3 were used as in-vitro model systems and the IC50 values of the indolequinones in all three cell lines were in the low nanomolar range. Indolequinones were also found to be efficient inducers of apoptosis in these cell lines at concentrations which induced growth inhibition. Selected indolequinones were screened against the NCI-60 cell line panel and their spectrum of activity was similar to established inhibitors of thioredoxin reductase. Indolequinones were therefore tested as inhibitors of thioredoxin reductase and were found to inhibit the enzyme in pancreatic cancer cells at concentrations equivalent to those inducing growth inhibitory effects. The mechanism of inhibition of thioredoxin reductase by the indolequinones was then studied in detail in cell-free systems using purified enzyme and the C-terminal selenocysteine of thioredoxin reductase was found to be the primary adduction site of the indolequinones using LC-MS analysis. Inhibition of thioredoxin reductase by indolequinones in pancreatic cancer cells resulted in a change of thioredoxin redox state and activation of the p38/JNK signaling pathway. Oxidized thioredoxin is known to activate apoptosis signal-regulating kinase 1 (ASK1), the upstream activator of p38/JNK in the MAPK signaling cascade, providing a potential mechanism for indolequinone-induced apoptosis. Our results demonstrate that thioredoxin reductase, p38 and JNK, are potential targets of anti-tumor indolequinones in pancreatic cancer cells (Supported by CA11441).

PS 1664 SDF-1β PROTECTS CARDIAC CELLS FROM PALMITATE-INDUCED NITROSATIVE STRESS-MEDIATED ER STRESS AND CELL DEATH THROUGH ACTIVATION OF AMPK-MEDIATED IL-6 EXCRETION.

Y. Zhao[2, 1], W. Li[2] and L. Cai[1, 2]. [1]*University of Louisville, Louisville, KY and* [2]*The Fist Hospital of Jilin University, Changchun, China.*

Elevated saturated free fatty acids are trigger for cardiac cell death,but its mechanisms remain unknown. Stromal cell derived factor-1beta(SDF-1β)was found to play cardiac protection,whether it protects the cardiac cells from lipotoxicity remains unknown.Using H9c2 cell line,the present study was undertaken to investigate apoptotic effect of Pal and possible protective effect of SDF-1β. Exposure of H9c2 cells to Pal at 62.5μM for 15h causes a significant apoptotic effect. This effect is mediated by endoplasmic reticulum (ER) stress associated cell death that is caused by NADPH oxidase (NOX)-activation associated nitrosative stress since Pal induces significant increases in 3-nitrotyrosine at 3–9h and ER stress at 6–9h after treatment.Inhibiting NOX-activation or scavenging superoxide and peroxynitrite with their specific inhibitors or scavenger abolishes Pal-induced ER stress and cell death.Inhibiting ER stress prevents cell death. Pretreatment with SDF-1β abolishes Pal-induced nitrosative damage,ER stress and cell death,along with up-regulation of AMPK-mediated IL-6 production. Direct addition of AMPK activator significantly prevents Pal-induced cell death and also increases IL-6 contents while inhibition of AMPK abolishes SDF-1β-mediated cardiac protection. To further define the role of AMPK-mediated IL-6 in cardiac protection,direct addition of recombinant IL-6 to cell cultures offers a significant prevent of Pal-induced ER-stress and cell death.These results suggest that cardiac apoptotic effect of Pal is mediated by NOX activation-triggered nitrosative damage and ER stress cell death pathway.Pretreatment with SDF-1β can significantly prevent Pal-induced cardiac cell death via activation of AMPK-mediated IL-6 to directly protect from cell death via inhibition of ER-stress.The finding that SDF-1β protects cardiac cell death from fatty acid via up-regulation of AMPK opens a new road for the research on cardiac protection by SDF-1β that provides cardiac protection independent of angiogenesis.

PS 1665 THE MECHANISMS OF PARAQUAT-INDUCED LUNG TOXICITY *IN VIVO* AND *IN VITRO.*

Y. Yang[1], D. Hung[1, 2], C. Chen[1, 3], H. Wu[4], K. Chen[4], C. Su[5], C. Huang[6] and Y. Chen[7]. [1]*Graduate Institute of Drug Safety, China Medical University, Taichung, Taiwan,* [2]*Toxicology Center, China Medical University Hospital, Taichung, Taiwan,* [3]*Department of Emergency, China Medical University Hospital, Taichung, Taiwan,* [4]*Department of Urology, China Medical University Hospital, Taichung, Taiwan,* [5]*Department of Otorhinolaryngology, Head and Neck Surgery, Changhua Christian Hospital, Changhua, Taiwan,* [6]*School of Chinese Medicine, China Medical University, Taichung, Taiwan and* [7]*Department of Physiology, China Medical University, Taichung, Taiwan.*

Paraquat (1,1'-dimethyl-4,4'-bipyridium dichloride) is an effective herbicide which is used all over the world. Paraquat induces fatal damage to multiple organs, especially lung. The mechanisms of cytotoxicity of paraquat are redox cycling and intracellular oxidative stress generation. In this study, we investigated the effects and the possible mechanisms of paraquat on the rat lung alveolar epithelial cell line (L2) and mouse lung tissue. In our study, L2 cells were exposed to paraquat (50–900μM) for 24 h. These results showed that cell viability were decreased, malondialdehyde (MDA) and ROS levels were increased in a dose-dependent manner after paraquat exposure for 24 h. Besides, paraquat increased proteins of Bax, cytosol apoptosis-inducing factor (AIF), cytosol Endonuclease G (Endo G) expression, and enhanced mitochondrial membrane potential depolarization. Furthermore, mitochondria-related apoptotic signals of mRNAs of Bax, Bid, p53, mdm-2 were increased in L2 cell after exposure to paraquat. In addition, mRNAs levels of endoplasmic reticulum stress (ER stress) related apoptotic signals of ATF4,ATF6,GRP78,GRP94,CHOP were significantly increased. In vivo, the MDA levels in plasma and lung tissues were dramatically raised after paraquat treatment, but these effects were reversed by antioxidant, N-acetyl-L-cysteine (NAC). These results suggest that paraquat induced alveolar epithelial cell death through a mitochondria- and ER stress-related pathway. These observations provide evidence that paraquat is an environmental risk factor for lung functional disorders and diseases.

PS 1666 ROLE OF CANNABINOID RECEPTORS IN CANNABIDIOL-MEDIATED APOPTOSIS IN LIPOPOLYSACCHARIDE(LPS) ACTIVATED B LYMPHOCYTES.

R. Rao, P. Nagarkatti and M. Nagarkatti. *University of South Carolina, Columbia, SC.*

Cannabinoids are biologically active components of marijuana (Cannabis sativa). Cannabinoids mediate their effects via the Cannabinoid receptors. Cannabinoid receptor 1 (CB1), although predominantly expressed in the brain, is also expressed in the cells of immune origin. Cannabinoid receptor 2 (CB2) however is primarily expressed in immune cells, with B lymphocytes expressing it in highest amounts. Cannabidiol (CBD) is a major non-psychoactive component of marijuana that exhibits anti-inflammatory properties. In the current study we investigated the effects of CBD on LPS activated B lymphocytes specifically its ability to induce apoptosis. We found that CBD at a concentration of 10-20 μM caused significant levels of apoptosis in CD19 positive B cells in vitro. B cells from CB1 knock out (KO) mice

showed similar levels of apoptosis as wild-type (WT) B cells. Furthermore, addition of CB2 select antagonist (SR144528) caused significant inhibition in apoptosis. Similarly, B cells from CB2 KO mice showed similar levels of sensitivity to CBD; However, addition of CB1 antagonist (SR141716) failed to inhibit the response because CB1 agonist appeared to act as a reverse agonist. We also investigated the role of transient receptor potential Vanilloid type 1 (TRPV1) which is known to be a ligand for CBD. Upon blocking the TRPV1 receptor, with Capsazepine (CPZ), a selective TRPV1 antagonist, CBD-induced apoptosis was not altereed. Pre-treatment of B lymphocytes in vitro with Caspase 3, 8 and 9 suggested a potential cross talk between the extrinsic and intrinsic pathways of apoptosis. Together, our data suggested that the anti-inflammatory properties of CBD can be attributed, at least in part, to apoptosis in activated B cells (Supported in part by NIH grants R01ES09098, P01AT003961, R01ES019313).

PS 1667 EFFECTS OF PBDES CONGENERS (BDE-209 AND BDE-47) ON HUMAN HEPATOMA CELLS (HEPG2) PROLIFERATION AND VIABILITY.

D. J. Dorta and A. O. de Souza. *Quimica, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - Universidade de Sao Paulo, Ribeirão Preto, SP, Brazil.*

INTRODUCTION: Polybrominated diphenyl ethers (PBDEs) are an important class of flame retardants used, nowadays, in a variety of electronic equipment. Factors such as their high lipophilicity and bioaccumulation have raised concerns about their toxic potential. In fact, recent studies reported that PBDEs have been shown toxic effects and they may vary depending on the studied specie. OBJETIVE: This work investigated the effects of the PBDE congeners -209 and -47 on the proliferation and viability of HepG2 cells. METHODOLOGY: Firstly, cell proliferation test was performed using the sulforhodamine B (SRB) colorimetric assay to evaluate the inhibitory potential of these congeners and then the cell viability test (MTT assay) was performed. In both tests, cells were incubated at 37°C, in an atmosphere containing 5% CO2 and 96% relative humidity for 24 hours before starting the treatment. Then, cells were treated with PBDEs (0.1μM, 0.5μM, 1μM, 5μM, 10μM and 25μM) for 24 and 48 hours. For the SRB assay, the dye was added and left at room temperature for 1h. Subsequently, the cells were washed twice and SRB attached to the cells membrane was extracted using Tris buffer, pH 8.0. The absorbance of the dye was then measured in a spectrophotometer at 540 nm wavelength. For the MTT assay, after the exposure time to the PBDEs, the cells were reincubated with MTT 0.5% solution for 3 hours for the formation of formazan crystals, which were solubilized with dimethyl sulfoxide and glycine buffer solution. The absorbance was analyzed at 570 nm wavelength. RESULTS: Both congeners evaluated are capable of inhibiting cell proliferation and significantly decrease cell viability at doses starting at 5μM, however in a bigger extension for BDE -47 CONCLUSIONS: According to these results, the PBDEs congeners -209 and -47 showed a significant potential for inhibit cell proliferation in HepG2 cells and induce cell death. Financial support: FAPESP, CAPES

PS 1668 HYDROXYL RADICALS MEDIATES CISPLATIN-INDUCED APOPTOSIS IN HUMAN HAIR FOLLICLES DERMAL PAPILLA CELLS AND KERATINOCYTES THROUGH BCL-2-DEPENDENT MECHANISM.

S. Luanpitpong[1, 3], P. Chanvorachote[3], V. Pongrakhananon[1, 3], L. Wang[2], U. Nimmannit[4] and Y. Rojanasakul[1]. *[1]West Virginia University, Morgantown, WV, [2]National Institute for Occupational Safety and Health, Morgantown, WV, [3]Chulalongkorn University, Bangkok, Thailand and [4]National Nanotechnology Center, Pathumthani, Thailand.*

Induction of massive apoptosis of hair follicle cells by chemotherapy has been implicated in the pathogenesis of chemotherapy-induced alopecia (CIA), but the underlying mechanisms of regulation are not well understood. The present study investigated the apoptotic effect of cisplatin in human hair follicle dermal papilla cells (HFDPC) and HaCaT keratinocytes, and determined the role of reactive oxygen species (ROS) in the process. Treatment of the cells with cisplatin induced ROS generation and a parallel increase in caspase activation and apoptotic cell death in HFDPC and HaCaT cells. Inhibition of ROS generation by antioxidants, N-acetyl cysteine and reduced glutathione, inhibited the apoptotic effect of cisplatin, indicating the role of ROS in the process. Studies using specific ROS scavengers further showed that hydroxyl radical, but not hydrogen peroxide or superoxide anion, is the primary oxidative species responsible for the apoptotic effect of cisplatin. The mechanism by which hydroxyl radical mediates the effect of cisplatin was shown to involve down-regulation of anti-apoptotic protein Bcl-2 through ubiquitin-proteasomal degradation. Bcl-2 was also shown to have a negative regulatory role on hydroxyl radical. Together, our results indicate an essential role of hydroxyl radical in cisplatin-induced cell death of hair follicle cells through Bcl-2 regulation. Since CIA is a major side effect of cisplatin and many other chemotherapeutic agents with no known effective treatments, the knowledge gained from this study could be useful in the design of preventive treatment strategies for CIA through localized therapy without negatively affecting the chemotherapy efficacy.

PS 1669 LC-ESI/MS REVEALS UNUSUAL OXYGENATED LYSO-PHOSPHATIDYLSERINES PRODUCED AFTER OXIDATION AND HYDROLYSIS BY PLASMA LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 OF SN-1, SN-2-DILINOLEOYL-PS.

V. A. Tyurin[1, 2], Y. Y. Tyurina[1, 2], C. H. Macphee[3] and V. E. Kagan[1, 2]. *[1]Center for Free Radical and Antioxidant Health, University of Pittsburgh, Pittsburgh, PA, [2]Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA and [3]GlaxoSmithKline, King of Prussia, PA.*

Oxidation and hydrolisis of phospholipids (PL), containing oxidizable residues in sn-2 position, play an essential role in the production of signaling molecules - oxygenated fatty acids (FA) and lyso-PL. The presence of oxidizable FA - in both sn-1 and sn-2 positions - may yield - upon oxidation and hydrolysis by phospholipases A2 (PLA2) - a large variety of oxidized species and non-oxidized species with potentially effective regulatory propensities. In particular, oxidative/hydrolytic metabolism of doubly polyunsaturated phosphatitdylserines (PS) may play a central role in the molecular mechanisms of apoptosis and clearance of apoptotic cells. We present the results on structural characterization of oxygenated molecular species of PS containing linoleic acid in sn-2 position (C18:0/C18:2) and in both sn-1 and sn-2 position (C18:2/C18:2) formed in cytochrome c/H2O2 driven reaction. Oxidation of PS(C18:2/C18:2) resulted in formation of products at m/z 798, 814, 830 and 846, which included OH, OOH, OH/OOH and 2OOH groups, respectively. We characterized PS hydrolysis products (lyso-PS and free FA, FFA) formed by lipoprotein-associated PLA2 - secreted type VIIA PLA2 and cytosolic type VIIB PLA2. PS was preferable substrate for VIIA PLA2. Accumulation of hydrolysis products was markedly greater after hydrolysis of oxidized PS than non-oxidized PS. The products were mainly oxygenated FFA and non-oxidized lyso-PS. Hydrolysis of oxidized PS (C18:2/C18:2), led to generation of a great variety of both oxygenated and non-oxygenated lyso-PS and FFA. We suggest that both oxidatively modified FFA and lyso-PS may represent important signals facilitating and regulating execution of apoptotic and phagocytotic programs essential for control of inflammation. Supported by OH008282, U19AI068021, HL70755, HL094488, GlaxoSmithKline.

PS 1670 GROWTH INHIBITION OF BENZO(A)PYRENE (BAP)-EXPOSED HT29 HUMAN COLON CANCER CELLS BY RESVERATROL.

A. C. Huderson, J. N. Myers and A. Ramesh. *Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN.*

The magnitude of human exposure to BaP through diet has generated a great deal of interest with regard to the association of ingested BaP with gastrointestinal carcinogenesis. Studies conducted in our laboratory have already shown that oral administration of BaP causes tumors in the colon of APC^{Min} mice. Given the fact that colon cancer ranks 3rd among cancer-related mortalities, and in the United States alone around 60,000 lives are lost every year to colon cancer, it is necessary to evaluate the effect of anti-carcinogenic compounds on colon cancer initiation and progression. In this study we investigated the anti-carcinogenic effects of resveratrol (RVT) on BaP-induced apoptosis in the human colon cancer cell line HT29. HT-29 cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% FBS and antibiotics (Penicillin/Streptomycin). Initially cells were revived by the addition of 1mL of HT-29 cell frozen stocks to 20mL of media. Every 2 -3 days the media was changed until the cell grew to be 80% confluent. After cells were observed to be healthy, DMSO stocks were made of the cells using 90% FBS and 10%DMSO. Cells were seeded onto a 6 well plate or a T75 flask at a density of 20,000cells/well or 500,000 cells respectively. The cells were then synchronized overnight by serum starvation (DMEM/F12 media, 1% FBS) method. Cells were treated with 5μM of BaP simultaneously with 40μM of RVT. After a 24, 48, 72, and 96 hour exposure period, cells were counted using Beckman coulter counter. Resveratrol alone or simultaneously with BaP caused growth inhibition of the HT29 cells when compared to the no treatment and DMSO treatment groups. A progressive decrease in growth inhibition of cells was noticed with an increase in duration of exposure to BaP and RVT. Studies are in progress to see if this preventive effect is concentration-dependent both for the anti-carcinogenic agent and the carcinogen. This study was funded by the NIH grants 5T32HL007735-12, 1RO1CA142845-01A1, 1RO3CA130112-01, and 1S11ES014156-01A1.

PS 1671 CYTOTOXICITY COMPARISON OF DIFFERENT ORGAN TOXICANTS IN ORGAN SPECIFIC CELL LINES.

Z. Lin, R. Swiss, Y. Will and M. Pletcher. *Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT.*

Attrition of drugs for safety liabilities in preclinical development as well as in late stage clinical trials continues to be a challenge for the pharmaceutical industry for financial and patient welfare reasons. Hepato, cardio and nephrotoxicity remain the primary forms of drug-induced injury leading to compound termination. As a result, in vitro cellular screening paradigms are being deployed as common practice in the early stages of drug discovery as a means to predict these end organ toxicities and prevent high-risk compounds from reaching in vivo evaluations. In this study we investigated if using a hepatic, cardiac and kidney-derived cell line could accurately predict specific organ toxicities. We tested 140 cardiotoxicants, 250 hepatotoxicants, and 60 nephrotoxicants in H9c2 (embryonic myocardium), HepG2 (hepatocellular carcinoma) and NRK-E52 (kidney proximal tubule) cell for their cytotoxicity . The experiments were designed and carried out in such way that all cells were seeded in the same density and exposed to the same diluted drugs at 11 different doses for 24 hours. Cell viability assays (ATP depletion) were carried out in triplicate using a Promega Cell viability assay kit and IC50 values were calculated for each drug x cell line treatment. The data showed that the majority of compounds, regardless of their different organ toxicities, had similar cytotoxic effects in all three cell lines. Only a very small percentage of compounds showed differential response in the cell lines and that differential response did not show a correlation between the known in vivo organ toxicity and the tissue of origin of the cell line. Our results suggest that different cell lines have relatively equal value in assessing general cytotoxicity and that organ toxicity cannot be accurately predicted using such a simple approach. Organ toxicity potentially results from compound accumulation in a particular tissue, metabolism and off target expression.

PS 1672 STORE-OPERATED CALCIUM CHANNELS PLAY A SIGNIFICANT ROLE IN ROS-INDUCED NECROTIC CELL DEATH.

F. M. Ramirez, R. Xie, S. S. Lau and T. J. Monks. *Southwest Environmental Health Sciences Center, Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.*

Oxidative stress is associated with many pathological conditions, including renal disease. The generation of reactive oxygen species (ROS) leads to DNA strand breaks, which activate the DNA repair enzyme poly (ADP-ribose) polymerase (PARP)-1. Hyper-activation of PARP-1 leads to NAD and ATP depletion and to increases in intracellular calcium concentrations (iCa2+), all of which contribute to necrotic cell death. The relationship between iCa2+ and oxidative stress induced was determined in renal proximal tubule epithelial cells (HK-2) exposed to the potent nephrotoxicant 2,3,5-tris-(glutathion-S-yl)hydroquinone (TGHQ). Inhibitors of calcium channels, such as the inner mitochondrial membrane calcium uniporter (MCU), the endoplasmic reticulum inositol 1,4,5-triphosphate receptor (IP3R), and store-operated calcium channels (SOCs) in the plasma membrane were used to pre-treat and co-treat HK-2 cells in the presence and absence of TGHQ in order to determine their role in cell death. Ru360 (10μM), a MCU inhibitor, and TMB-8 (100μM), an IP3R inhibitor, had no effect on TGHQ-induced cell death. In contrast, 2-APB (100μM), a SOCs inhibitor, maintained cell viability and the mitochondrial membrane potential to ~60% of controls. Overall, the data reveal that although the MCU and the IP3R do not appear to play a direct role in TGHQ-induced cell death, SOCs have a significant role in response to oxidative stress. We are currently investigating the relationship between PARP-1 and SOCs in TGHQ-induced cell death via direct measurement of NAD and calcium imaging. (P30ES006694)

PS 1673 PROTECTION OF HYDROQUINONE-INDUCED APOPTOSIS BY FAU IS MEDIATED BY NQO1 IN W7.2 MOUSE THYMOMA CELLS.

S. H. Inayat-Hussain[1], L. E. Siew[1], M. K. Chan[1], N. F. Rajab[1], D. Ross[2] and G. T. Williams[3]. *[1]Environmental Health Program, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia, [2]School of Pharmacy, University of Colorado at Denver, Denver, CO and [3]Institute for Science and Technology in Medicine and School of Life Sciences, Keele University, Keele, United Kingdom.*

Hydroquinone (HQ) is a major metabolite of benzene. However, the role of fau in HQ-induced apoptosis is still poorly defined. fau (Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV)-associated ubiquitously expressed gene) was identified using functional expression cloning as a potential tumor suppressor gene. In this study, downregulation of fau by the antisense orientation of fau (rfau) in W7.2 cells was investigated whether it offered any protection on HQ induced DNA damage, reactive oxygen species (ROS) and apoptosis. Our data showed that HQ induced apoptosis via DNA damage and ROS was abrogated using a stable clone of W7.2 rfau cells. Further investigation revealed that there was an elevation of NAD(P)H: quinone oxidoreductase 1 (NQO1) protein in W7.2 rfau cells. Importantly, inhibition of NQO1 enzymatic activity using MAC220, a specific inhibitor and knockdown of NQO1 by siRNA rendered the cells to be susceptible to HQ induced toxicity. Taken together, this study suggests that cytoprotection of HQ toxicity by downregulation of fau is mediated by NQO1 and there is a possible role for fau in the manifestation of benzene toxicity.

PS 1674 AFLATOXIN PREVALENCE AND HEPATOCELLULAR CARCINOMA IN GHANA: A REGIONAL ANALYSIS.

N. J. Mitchell[1], P. E. Jolly[2], C. M. Jolly[3], A. Marroquin-Cardona[1] and T. D. Phillips[1]. *[1]College of Veterinary Medicine, Texas A&M University, College Station, TX, [2]Department of Epidemiology, School of Public Health, University of Alabama at Birmingham, Birmingham, AL and [3]Department of Agricultural Economics and Rural Sociology, Auburn University, Auburn, AL.*

Cancer incidence is rising in Africa with approximately 650,000 people developing cancer annually with an 80% mortality rate. Hepatocellular carcinoma (HCC) is one of the most common malignancies in Ghanaian men and women. Mortality among Ghanaian patients with HCC accounts for 21.15% and 10.9% of all cancer related deaths respectivley. Multiple factors play a role in the etiology of HCC; one major risk factor is chronic exposure to low levels of aflatoxins (AFs) from the diet. It is well established that the predominant congener, aflatoxin B_1 (AFB_1), is one of the most potent naturally-occurring hepatocarcinogens in humans. A well-defined biomarker of exposure, aflatoxin M_1 (AFM_1), has been correlated with an elevated risk of HCC in several human populations. In this study, urinary AFM_1 biomarkers from 324 Ghanaians (representing the Ashanti, Greater Accra and Central regions) were analyzed for future possible correlations with cancer prevalence and dietary intake of AFs. Biomarkers were extracted from urine using AflaTest® immunoaffinty columns and measured using HPLC analysis with fluorescence detection. AFM_1 was detected in 43.4% of the urines with values ranging from 233.2 pg/dl to 818,769 pg/dl urine. The calculated median value was 3,313.4 pg/dl urine. Differences were noted in the percent mortality rates from HCC in each region. Specific values were as follows; Ashanti 25.18%, Greater Accra 43.55%, and Central 9.36%. AFs exposure and HCC incidence is an ongoing public health problem in Ghana, especially in the Ashanti and Greater Accra regions where HCC mortality rate is the highest. Determination of the prevalence of AFs in different regions of Ghana is an important tool to assess the predominant sources of exposure, as well as those populations that are most at risk for HCC due to AFs.

PS 1675 CYTOCHROME P4501A PROMOTER POLYMORPHISMS AND ACTIVITY IN NATURAL POPULATIONS ADAPTED TO CHRONIC POLLUTION.

L. Williams[1] and M. Oleksiak[2]. *[1]Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC and [2]University of Miami, Miami, FL.*

Cytochrome P4501A (CYP1A) has been shown to be refractory to prototypic inducers in populations of the estuarine minnow, Fundulus heteroclitus, adapted to chronic anthropogenic pollution. Two single nucleotide polymorphisms (SNPs) in the promoter and first intron of the CYP1A promoter were previously found to be under selection, indicating that natural selection was acting on the CYP1A promoter or loci linked to these SNPs. In order to understand the role of the CYP1A promoter and these selectively important SNPs, the promoter and first intron and exon were sequenced in multiple individuals in one polluted (New Bedford Harbor) population resistant to PCBs and other anthropogenic contaminants and two reference populations north and south of the polluted site. The CYP1A promoter was extremely variable (an average of 21% of the promoter nucleotides varied among all populations) and there were no fixed differences between populations. The inducibility of the promoter was also explored; the promoter was induced by a prototypic PAH, 3-methylcholanthrene, in a dose-dependent manner. When promoters from multiple individuals per population were tested, the CYP1A promoter was significantly induced in the polluted New Bedford Harbor population as compared to both reference populations. This is in contrast to CYP1A expression in vivo which is refractory to induction in New Bedford Harbor individuals. Overall, the variation in the promoter does not explain the variation in in vitro promoter expression in all tested populations. These results indicate that the underlying mechanism for the in vivo transcriptional phenotype may lay further upstream or downstream of the CYP1A promoter region which was sequenced (1600bp), or involve proteins which were not available in the cell line (top minnow) in which the transfection assays were completed.

PS 1676 TWO BIOMARKERS FOR EVALUATION OF ORGANOPHOSPHATE (OP) POISONING IN HUMANS.

G. H. DeOliveira[1], G. L. Emerick[1], K. A. Belaz[2], M. Gonçalves[1] and R. V. Oliveira[2]. [1]*Natural Actives Principles and Toxicology, UNESP Sao Paulo State University, Araraquara, Sao Paulo, Brazil and* [2]*Department of Chemistry, University Federal of Sao Carlos, Sao Carlos, Sao Paulo, Brazil.*

World Health Organization recognizes that the activity of acetylcholinesterase (AChE) is a useful preventative biomarker to overexposure to OP. On the other hand, in spite of the neuropathy target esterase (NTE) had been identified by Johnson (1969) as a target of neuropathy induced OP, we did not have knowledge of the normal value of it activity into human lymphocyte. Therefore, this work aimed to establish reference value for normal activity of AChE in erythrocytes and NTE in lymphocytes (LNTE) in blood of donors of the hemocenter of Araraquara, SP Brazil. Besides all the requirements that a blood donor must have, we applied a questionnaire prior to a screening and know more about some habits of each volunteer. In a total of 45 volunteers we conducted the following key questions: Is there any industry near your home? Do you smoke? Do you have difficulty remembering things? Are you taking any medicine? Then the venous blood was collected in Vacutainer tubes with sodium heparin. We made the separation of lymphocytes from 2.5 ml of blood using the Histopaque-1077 and for the separation of erythrocytes we centrifuged 0.5 ml of blood at 2500 rpm for 5 min. The values obtained for AChE and LNTE were 6.82 ± 1.29 μmol/min/g proteins and 7.02 ± 1.85 μmol/min/g proteins, respectively. It was made a separation between men and women and 77.8% of the volunteers were men with values of 6.8 ± 1.2 μmol/min/g protein for AChE and 7.3 ± 1.78 μmol/min/g protein for LNTE. The women had values of 6.23 ± 1.01 μmol/min/g proteins for AChE and 6.64 ± 1.89 μmol/min/g proteins for LNTE. We conclude that these data may be useful in future work aimed at delineating the activity of NTE as a biomarker of effect for agricultural workers from exposure to neuropathic OP. Human Ethics Committee, Resolution: 31/2009. Financial support: FAPESP 2009/51048-8.

PS 1677 GLOBAL GENE EXPRESSION CHANGES BY HUMAN UROEPITHELIAL CELLS EXPOSED TO LOW-LEVEL MONOMETHYLARSONOUS ACID.

X. Zheng, P. Novak, S. M. Wnek, M. K. Medeirous, V. Chyan and J. A. Gandolfi. *Pharmacology/Toxicology, University of Arizona, Tucson, AZ.*

Global gene expression effects of a 3 month exposure of low concentrations of monomethylarsonous acid [MMA(III), a metabolite of arsenate] to human uroepithelial (UROtsa) cells was investigated. To identify the molecular targets of MMA(III) in human cells, total RNA was isolated from UROtsa cells exposed for 0-3 months to 50 nM MMA(III) and then an Affymetrix Human gene array (28K genes) was used to carry out the cDNA microarray assay. Small, progressive changes are seen with some genes over at 1-2 month exposure to MMA(III) followed by a much larger change between 2-3 month of exposure. Using a log2 cut-off, there are 168 up-regulated genes and 442 down-regulated genes in response to 3 month exposure. The gene changes at 3 month of exposure are coincident with these cells having an increase in proliferation, DNA damage, and eventual capabilities to grown in soft agar and form tumors in immuno-compromised mice. Six up-regulated genes and six down-regulated genes were selected to validate the array data and used as "marker" genes related to cellular and molecular functions: TMPRSS11A and KRT7 for cell cycle, CXCR4 and TGFBI for cell proliferation, PDGFRA and IRF1for cell differentiation, CTNND2 and ICAM1 for cell adhesion, CRYAB and DUSP1 for oxidative stress, and ERCC2 and DCLRE1C for DNA repair. In general, these genes demonstrated a time-dependent change starting at 1 month exposure to 50 nM MMA(III) with greater alterations seen at the 3-month time point. More detailed chronological assessment of the global gene alterations along with their correlation to phenotypic changes should provide clues to the underlying the mechanisms of human diseases related to chronic arsenic exposure. (NIEHS 04940 and 06694).

PS 1678 METABOLIC PROFILING OF SERUM FROM RATS DOSED WITH ACETAMINOPHEN AND CARBON TETRACHLORIDE.

R. D. Beger, L. Pence, L. K. Schnackenberg, J. Sun, T. Schmitt, X. Yang, J. Greenhaw, S. Bhattacharyya, W. Salminen and D. L. Mendrick. *Division of Systems Biology, NCTR, U.S. FDA, Jefferson, AR.*

Hepatotoxicity is a major reason that drugs are withdrawn post-market or have a black box warning label. Current clinical chemistry biomarkers of liver injury are not always adequate in terms of specificity; therefore new specific biomarkers of liver injury are needed. Metabolic profiling methods were used to find and evaluate biomarkers of liver toxicity with two classic hepatotoxins. Male Sprague-Dawley rats were dosed with acetaminophen (APAP) or carbon tetrachloride (CCl4) and blood samples were collected at 6 hours, 24 hours, and 3 days after dosing. Rats were dosed with either a single dose of 0 (0.5% methylcellulose), 100 or 1250 mg/kg APAP or given 0 (corn oil), 50 or 2000 mg/kg CCl4 for 3 consecutive days. Rats were sacrificed at 6 and 24 hours and 3 days and tissues collected for histopathology. Nuclear magnetic resonance, gas chromatography coupled to mass spectrometry and ultra performance liquid chromatography coupled to mass spectrometry were used to profile bile acids and to discover new biomarkers of hepatotoxicity. Histopathology showed minimal to moderate centrilobular necrosis at 24 hours after high doses of APAP. For CCl4, histopathology showed mild to marked centrilobular necrosis at 24 hours after high dosing and minimal to mild centilobular necrosis at 3 days after dosing. Serum levels of cholic acid and glycocholic acid were significantly higher at 24 hours after a high dose of APAP or CCl4. Taurocholic acid was increased at 6 and 24 hours after a high dose of CCl4. Metabolic profiling of blood samples from rats dosed separately with APAP or CCl4 was successful in finding potential biomarkers of liver toxicity. Several bile acids were elevated before traditional clinical chemistry markers of liver injury. More liver toxicity studies are needed to determine whether these potential biomarkers are more specific for liver injury than current clinical chemistry biomarkers.

PS 1679 CHARACTERIZATION OF PEPTIDE ADDUCTS OF REACTIVE NAPHTHALENE METABOLITES IN THE URINE OF MALE MICE BY MASS SPECTROMETRY.

N. Pham[2, 1], A. Buckpitt[1, 2], D. Morin[1] and W. T. Jewell[2]. [1]*Veterinary Medicine, Molecular Biosciences, University of California Davis, Davis, CA and* [2]*Pharmacology and Toxicology, University of California Davis, Davis, CA.*

Naphthalene, a volatile polycyclic aromatic hydrocarbon generated during combustion, is metabolized to reactive metabolites which bind covalently to proteins in vivo and in vitro. In general, covalent binding levels correlate with the species and tissue selective toxicity associated with naphthalene. The substantial differences in susceptibility to naphthalene toxicity in different rodent species combined with widespread human exposure supports the need to understand whether this compound poses a risk for humans. Although covalent adducts with hemoglobin and albumin have been used as surrogates for examining the generation of reactive metabolites, their measurements have not lead to a clearly established relationship between adduct levels and toxicity. The hypothesis being tested in these studies is that the formation of critical adducts in target tissues can be followed by measuring downstream products, specifically adducted peptides, in the urine. To establish methodology for measuring adducted peptides in the urine, 24 hr urine samples were obtained from mice treated with 50/50 mixtures of 2H/1H-naphthalene either IP or by inhalation. Separation of water soluble metabolites and adducted peptides by reverse phase HPLC followed by HR-MS revealed the presence of numerous putative peptide adducts with the expected isotopic ratios and molecular masses which varied from 600 to 900Da. Partial sequences obtained for some of these entities and differences in chromatographic/mass spectral characteristics with known water soluble metabolites of naphthalene further confirmed the presence of urinary peptide adducts. The urine remains a relatively unexplored biofluid for investigation of protein/peptide adducts that could act as markers of metabolic processes key to the mechanisms of toxicity of numerous chemicals which undergo metabolic activation. Characterization and identification of these potential candidates is ongoing. Supported by NIEHS 04311 and 04699.

PS 1680 INSECTICIDE RESIDUES AND THEIR SELECTED BIOMARKERS IN PRODUCE: FIELD STUDIES USING MALATHION AND FENPROPATHRIN IN STRAWBERRIES.

L. Chen[1], Z. Chen[1, 2], Y. Liu[1], T. Lopez[1, 2], G. Sankaran[1, 2], H. Vega[1] and R. I. Krieger[1, 2]. [1]*Personal Chemical Exposure Program, Department of Entomology, University of California, Riverside, CA and* [2]*Environmental Toxicology Graduate Program, University of California, Riverside, CA.*

Trace levels of pesticides used in conventional or organic crop protection and degradation products may occur in produce. Low level human dietary and non-occupational urine biomonitoring studies may be confounded by preformed pesticide biomarkers used to infer potential human pesticide exposure. This research investigated the formation of potential urine biomarkers in strawberries under normal field conditions in Santa Maria, CA. Malathion and fenpropathrin insecticides were sprayed (lb/A) in August, 2010. Residue and biomarker analysis using

GC/MS was performed on 8 one-pound samples using matrix-matched standards. The LOQs were 0.005 ug/g and recoveries of spiked biomarkers at 0.01, 0.1 and 1 ug/g of each analyte ranged from 79 to 101%. Strawberries on d 4 contained 0.23 and 0.46 ug/g malathion and fenpropathrin, respectively. Malathion and malaoxon, malathion monoacid and malathion diacid dissipated with t1/2s of 3.4, 7.4, 7.5 and 6.5 d, respectively, during a 10 d study period. The derivatives were more persistent than malathion, and the malathion diacid was most abundant at each sampling. Fenpropathrin and 3-phenoxybenzoic acid (PBA) dissipated with t1/2s of 5.9 and 2.7 d, respectively. The PBA levels ranged from 0.087 to 0.021 ug/g. Malathion and fenpropathrin residues were always below established residue tolerances. Potential biomarkers of pesticide exposure were present in every sample. The absorption and excretion of preformed biomarkers by consumers would falsely indicate pesticide exposure when used to reconstruct dose for risk characterization.

PS 1681 QUANTITATION OF THE BENZO[A]PYRENE (B[A]P) METABOLOME BY A STABLE ISOTOPE DILUTION TANDEM MASS SPECTROMETRY METHOD AND ITS APPLICATION TO HUMAN BRONCHOALVEOLAR CELLS.

D. Lu[1, 2], R. Harvey[4], I. Blair[1, 3] and T. Penning[1, 2]. *[1]Department of Pharmacology, University of Pennsylvania, Philadelphia, PA, [2]Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA, [3]Center for Cancer Pharmacology, University of Pennsylvania, Philadelphia, PA and [4]The Ben May Department for Cancer Research, University of Chicago, Chicago, IL.*

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants and are carcinogenic in multiple organs and species. Recently, benzo[a]pyrene (B[a]P), a representative PAH has been designated as a Group 1 human carcinogen by the International Agency for Research on Cancer. B[a]P requires metabolic activation to exhibit its toxicity and carcinogenicity. The three major metabolic pathways involved include formation of radical cations (peroxidase mediated), diol-epoxides (P450 mediated) and *o*-quinones (aldo-keto reductase mediated). We want to determine the contributions of these different metabolic pathways to cancer induction and prevention by quantitating signature metabolites from each metabolic pathway. By successfully designing and synthesizing a library of [$^{13}C_4$]-labeled B[a]P metabolite internal standards, we developed a sensitive stable isotope dilution atmospheric pressure chemical ionization tandem mass spectrometry method to quantitate B[a]P metabolites. The LOD of the generally accepted biomarkers for B[a]P exposure, such as B[a]P-7,8-dihydrodiol and 3-OH-B[a]P, by this method was 1.5 fmol on column and their LOQ was 6 fmol on column. This method exhibits a 500 fold increased sensitivity compared with a method using HPLC-radiometric detection, which has a LOD of 1 pmol on column. This method was then applied to study the B[a]P metabolome in human bronchoalveolar H358 cells in the presence and absence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a cytochrome P450 inducer. The sensitivity of the method should permit the metabolism of B[a]P to be measured in any setting and could also be used for biomonitoring human exposure to B[a]P. [Supported by 1P30-ES013508 and 1R01-ES-15857 to TMP]

PS 1682 MALDI IMAGING OF PROSTATE CANCER TISSUE TOWARD VALIDATION OF BIOMARKER IDENTIFICATION.

C. M. Hattan[1], N. J. Mastrandrea[1], J. M. C Gard[2], R. Nagle[2], T. J. Monks[1] and S. S. Lau[1]. *[1]Southwest Environmental Health Sciences Center, Department of Pharmacology/Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ and [2]College of Medicine, University of Arizona, Tucson, AZ.*

Prostate-serum antigen, a biomarker for prostate cancer detection, does not differentiate between benign and malignant cancers. Mass-spectrometry-based techniques permit the identification not only of specific proteins unique to diseased tissue, but also whether such proteins are present in a form amenable to therapeutic intervention (whether they are "on" or "off" via posttranslational modifications [PTM]). MALDI tissue and drug imaging of cancer cells were used to identify PTM of signaling proteins. Utilizing a PTEN deficient human prostate cancer cell line, LNCaP, we examined the expression and PTM of eukaryotic initiation factor 4E binding protein 1 (4EBP1) as a potential biomarker for prostate cancer. 4EBP1 is a key protein involved in the PI3K/AKT and mTOR signaling pathways, it is over-expressed in certain cancers, and hyper-phosphorylated during prostate cancer progression. IP of 4EBP1 followed by MALDI, LC-MS/MS analysis and western blotting demonstrated 4EBP1 contained multiple phosphorylation sites. Regulation of EGFR/RTKs by Tarceva and MP470 both modulate downstream Raf/MEK/ERK and/or mTOR/4EBP1 signaling pathways. Treatment of LNCaP cells with Tarceva/MP470 completely abolished 4EBP1 phosphorylation, as determined by 4EBP1 IP & MALDI analysis. Moreover, MALDI imaging and IHC staining permitted spatial localization of signaling proteins on frozen human prostate cancer tissue. Thus, a detailed profiling of "operative" growth regulatory pathways in individual patient tumor tissue is now possible with current tissue and drug imaging technology. Such information may be essential to guide the selection of pathway-selective therapy to ensure optimal tumor cell killing, and patient survival. Such "pathway profiling" of the primary tumor may also be of benefit when deciding on the selection of targeted therapy for metastatic sites. (GM070890, TGen Pilot Project, Ventana/Roche, P30ES006694, T32ES016652, AstraZeneca Studentship)

PS 1683 MOLECULAR DOSIMETRY OF N²-HYDROXYMETHYL-dG ADDUCTS FOLLOWING FORMALDEHYDE EXPOSURE TO NON-HUMAN PRIMATES.

B. C. Moeller[1], K. Lu[2], M. Doyle-Eisele[3], J. McDonald[3], A. Gigliotti[3] and J. A. Swenberg[1, 2]. *[1]Curriculum in Toxicology, University of North Carolina - Chapel Hill, Chapel Hill, NC, [2]Department of Environmental Sciences and Engineering, University of North Carolina – Chapel Hill, Chapel Hill, NC and [3]Lovelace Respiratory Research Institute, Albuquerque, NM.*

Formaldehyde (FA) is a ubiquitous chemical that is produced endogenously and used in industrial activities. It is a known carcinogen, causing squamous cell carcinoma in nasal tissue of rats, nasopharyngeal cancer in humans and has limited evidence from epidemiological studies for an association with myeloid leukemia. FA is highly reactive and can adduct to both proteins and DNA forming FA-DNA monoadducts and DNA-protein cross-links. A sensitive and selective nano-LC-MS/MS method was used to differentiate between exogenous and endogenous FA-monoadducts following whole-body exposure with [$^{13}CD_2$]-formaldehyde at 2 or 6 ppm to cynomolgus macaque (Macaca fascicularis) for 2 days (6 hours per day). Following completion of the exposures, the animals were immediately euthanized and tissues collected. For adduct quantitation, DNA from nasal maxilla-turbinates and femoral bone marrow were isolated, reduced with $NaCNBH_3$ and digested prior to HPLC fractionation. N^2-$HOCH_2$-dG and its corresponding $^{13}CD_2$ exogenous adduct were quantified by nano-LC-ESI-MS/MS. In the nose, endogenous N^2-$HOCH_2$-dG adducts were found in all animals studied at 2.24 ± 0.50 adducts/10^7 dG, while exogenous N^2-$HO^{13}CD_2$-dG was present at 0.25 ± 0.04 and 0.41 ± 0.05 adducts/10^7 dG at the 2 and 6 ppm exposures, respectively. In bone marrow, no exogenous adducts were detected using 5-fold more DNA, while endogenous adducts were present in all animals. Additional tissues were collected and ongoing studies are continuing to investigate adducts in several additional tissues including: lung, trachea, white blood cells, thymus, spleen, liver and brain. These data will provide critical data for science-based risk assessment of FA exposure. It demonstrates that primates form 5-10 fold lower amounts of exogenous DNA damage in nasal tissue than is formed in rats.

PS 1684 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN RESPONSIVE TRANSCRIPTS IN AVIAN EMBRYONIC LIVER, IDENTIFIED BY LARGE-SCALE DIFFERENTIAL DISPLAY SYSTEM WITH VERTEBRATE-COMMON DEGENERATE OLIGONUCLEOTIDE PRIMERS.

H. Teraoka[1], S. Ito[1], N. Ukai[1], E. Kim[2, 3], H. Iwata[3], T. Kitazawa[1], T. Hiraga[1] and D. Endoh[1]. *[1]School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan, [2]Department of Biology, Kyung Hee University, Seoul, Republic of Korea and [3]Center for Marine Environmental Studies, Ehime University, Matsuyama, Ehime, Japan.*

Research of possible impacts of toxic substances on gene expressions in a variety of organisms in the environment has been hampered due to lack of detailed molecular information required. In this study, we developed a large-scale differential display systems with vertebrates-common primer sets, based on degenerate oligonucleotide-primed PCR (DOP-PCR). Inverse repeat motif of less than 8 mer was found in most transcripts (78.3-99.8%) of 8 vertebrate species from fish to primates. Furthermore, more than ten thousand 8-mer inverse repeat motifs were commonly recognized in transcripts of 8 vertebrates. Among these inverse repeat motifs, 275 common motifs were selected to cover roughly 30% of transcripts throughout 8 vertebrates. 275 DOP primers were prepared with connected with several degenerate sequences and linker sequence to 5' end of 8-mer inverse repeat

motifs selected. This system was applied to detect genes responsive to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in livers of two avian species in ovo, domestic fowl as one of 8 species used for primer design and common cormorant as wild birds without detailed molecular background. We found 14 coding genes, 1 unknown coding gene and 1 non-coding transcript in embryonic livers of domestic fowl and common cormorant, most of which were newly identified as TCDD-responsive transcripts throughout any vertebrates. Transcripts other than target of the DOP primer set were frequently cloned due to mismatch amplification with several base pairs in 8-mer motif, showing that more than 30% transcripts could be targeted. These results highlighted the usefulness of systematically designed large-scale differential display system to search genes responsive to chemicals for any vertebrates without molecular and toxicological information in advance.

PS 1685 IS TRICHLOROETHYLENE-INDUCED FORMIC ACIDURIA A BIOMARKER OF EXPOSURE RATHER THAN A SIGN OF TOXICITY?

E. A. Lock, N. Yaqoob and A. Evans. *School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom.*

Trichloroethylene (TCE) is a widely used industrial solvent and is a common pollutant of air, soil and water. Administration of large oral doses of TCE to F344 male rats causes formic aciduria (Green et al.,(1998) Toxicology. 127, 39–47). In this study we have confirmed this finding and studied the dose-response relationship and the effect of TCE metabolites on formic acid excretion. Male F344 rats were orally dosed, daily for 3 days, with TCE (5ml/kg in corn oil) over a range of doses from 1000 to 2mg/kg/day and placed in metabolism cages for the collection of urine daily over 4 days. Day 0 urine was prior to dosing. Urine was collected over Na azide and then analysed for pH and formic acid by 1H-NMR spectroscopy using TSP as a standard. Studies were approved by the UK Home Office with 3-5 animals per dose. Some rats were dosed with 1- aminobenzotriazole (ABT), a suicide inhibitor of CYP450 (100mg/kg ip) 4h before TCE or with trichloroethanol (TCE-OH) or trichloroacetic acid (TCA) at 16mg/kg and the urine collected as above. Undosed rats excreted about 1mg formic acid/24h while rats dosed with TCE at 1000mg/kg excreted on average 10mg/24h on day1, 17mg/24h on day2 and 20mg/kg on day3. Decreasing doses of TCE down to 12mg/kg showed a similar response, which then reduced such that at 2mg/kg/day (the lowest dose tested) about 5mg formic acid/24h/day was detected. Urinary pH was more acidic at dose > 16mg/kg/day. Pretreatment with ABT followed by TCE at 16mg/kg prevented the increase in formic acid excretion. While dosing with TCE-OH and TCA caused formic aciduria which was most marked following TCA exposure. These studies shown for the first time that TCE-induced formic aciduria occurs at doses below those which produce toxicity and suggests this may be a useful biomarker of exposure. The response requires cytochrome P450 dependant metabolism of TCE, which is consistent with the finding that both major metabolites of TCE produce formic aciduria.

PS 1686 VALIDATION OF A PORTABLE X-RAY FLUORESCENCE (XRF) TECHNOLOGY TO QUANTIFY LEAD IN BONE *IN VIVO.*

S. Sanchez[1], M. G. Weisskopf[2] and L. H. Nie[1]. [1]*School of Health Sciences, Purdue University, West Lafayette, IN and* [2]*Harvard School of Public Health, Boston, MA.*

Lead is a ubiquitous toxicant in the environment. Bone lead represents cumulative lead exposure and is a better biomarker to assess association between lead and some adverse health outcomes especially the health outcomes related to long-term chronic lead exposures. Bone lead measured by a K x-ray fluorescence (KXRF) technology has been used as a biomarker for epidemiology and toxicology study on lead for over two decades. However, there are few labs worldwide possess the KXRF technology because of its infrastructural demands. We investigated the feasibility and methodology of the development of a portable in vivo x-ray fluorescence (XRF) bone lead quantification system in our previous studies (Nie LH et al. in press). In this study, we validate this technology with lead doped bone-equivalent phantoms, blank soft tissue-equivalent phantoms, and human cadaver bones. Because the technology depends heavily on the thickness of the soft tissue which overlays the bone, we tested the accuracy and precision of this technology at different soft tissue thicknesses. We also tested the reproducibility of the technology with continuous measurements and measurements at different times. The reproducibility of the technology with human cadaver bones was also tested. Two main results were obtained from this study: a) both accuracy and precision for this technology degrades with the increase of the tissue thickness; however, the results are not significantly different from the true value for tissue thicknesses less than 4 mm; b) the results are highly reproducible with both phantoms and the human cadaver bones. In conclusion, the portable XRF technology we developed is a valid technology for in vivo quantification of lead in bone. Nie LH, Sanchez S, Newton K, Grodzins L, Cleveland R, Weisskopf MG. In Vivo Quantification of Lead in Bone with a Portable XRF System – Methodology and Feasibility, Physics in Medicine and Biology, In Press

PS 1687 USE OF GLOBIN ADDUCTS FOR 1, 3-BUTADIENE INTERNAL DOSIMETRY THAT REFLECTS EXPOSURE AND METABOLISM.

N. K. Bordeerat[1], S. Carro[1], L. Collins[1], W. Bodnar[1], P. Upton[1], V. Walker[2] and J. A. Swenberg[1]. [1]*Environmental Science and Engineering, The University of North Carolina at Chapel Hill, Chapel Hill, NC and* [2]*University of Vermont, Burlington, VT.*

Butadiene (BD) carcinogenicity in rodents shows gender, species and concentration dependency, making the extrapolation of animal results to humans complex. BD is a multispecies carcinogen, with mice being a much more sensitive species than rats. This is considered to be related to the metabolism of BD to its epoxy metabolites. The mutagenic potency of individual epoxides varies up to 200-fold, with diepoxide being the most mutagenic. For accurate risk assessment it is important to elucidate species differences in the internal formation of the individual epoxides in order to assign the relative risks associated with their different mutagenic potencies. N-terminal globin adducts have been applied for measurements of the formation of BD derived epoxides. In this study, the formation of each epoxide was evaluated in globin samples from both genders of rodents exposed to BD by inhalation for 10 days. The number of globin adducts were then converted into EB dose equivalents. These were calculated on the basis of the combined internal dose (globin adducts) and the relative genotoxic potency of the respective epoxides inferred from the efficiency of inducing mutations at the Hprt locus. Based on the EB equivalent, higher exposures formed lower amounts per ppm BD. This indicates that metabolism of BD to epoxides is most effective at low exposures. The EB equivalent for mice was about 40-fold higher than rats at similar exposures. No gender differences were noted in globin adducts of rodents at all exposures. As such, EB dose equivalents provide quantitative data on biomarkers of exposure that can be compared with DNA adducts and mutations. Since these data demonstrated that there were no gender differences in metabolism, yet female rats and mice have been shown to have increased amounts of DNA cross-links and Hprt mutations, the collective data suggest a gender difference in repair of DNA cross-links in females that results in increased numbers of mutations.

PS 1688 AFLATOXIN EXPOSURE IN RURAL RESIDENTS OF BURKINA FASO, WEST AFRICA.

P. A. Nikiema, G. Qian, L. Tang, J. H. Williams and J. Wang. *University of Georgia, Athens, GA.*

Aflatoxins (AF) are significant toxic contaminants of food. AF exposure and associated human diseases are serious global public health concerns, especially for the developing world. To establish a global network for assessment of AF exposure in high-risk populations, a two-wave cross-sectional study (n=812) and an intervention study (n=493) were conducted in rural areas of Burkina Faso. Dietary survey was conducted for assessing dietary AF exposure and blood samples were drawn for AF biomarker evaluation. Levels of serum AFB1-lysine adduct (AFB-Lys) were measured by HPLC-fluorescence. Samples collected in Sep-Oct wave showed significant differences ($p<0.001$) on the median AFB-Lys levels among villages, with higher adduct level in Sourkoudingan (n=160; median=17.5; range: 2.8-627.8 pg/mg albumin), as compared to Campela (n=184; median=10.0; range: 0.5-978.7 pg/mg albumin) and Boende (n=138; median=10.2; range: 0.8-135.6 pg/mg albumin). The different exposure patterns were also found in samples collected in Apr-May wave from the same villages, with Campela (n=106; median 12.1; range: 0.5-192.4 pg/mg albumin) having higher adduct levels ($p<0.001$), as compared to Sourkoudingan (n=115; median=6.4; range: 0.4-493.9 pg/mg albumin) and Boende (n=109; median=6.9; range: 1.5-174.0 pg/mg albumin). The intervention study was conducted in villages Badala, as the control, and Diofoloma as intervened. Intervention protocol used was education on drying, sorting, and storage for ground nuts. To evaluate the efficacy, levels of AFB-Lys in blood samples collected before or after 10-month intervention. The median levels of AFB-Lys adduct are similar in both villages before the intervention (13.51 vs. 13.87 pg/mg albumin). After 10-month intervention, the median AFB-Lys level decreased about 14.6% in intervened group (14.18 pg/mg albumin) as compared to the control group (16.60 pg/mg albumin) with no statistical significance ($p=0.774$). Results of these two

studies showed that AF exposure in rural residents of Burkina Faso is high and other effective and targeted intervention strategy, in addition to education, are urgently needed.

PS 1689 EMPLOYING A NEW ANALYTICAL METHOD FOR MEASURING THE CYANIDE METABOLITE, 2-AMINOTHIAZOLINE-4-CARBOXYLIC ACID, IN BIOLOGICAL SAMPLES.

J. C. Yu[2], I. Petrikovics[1], S. Martin[1, 2], J. Nasr[1], D. Thompson[1] and S. Holmes[1]. [1]*Chemistry, Sam Houston State University, Huntsville, TX and* [2]*Forensic Science, Sam Houston State University, Huntsville, TX.*

A new, sensitive method was developed for the identification and quantification of 2-aminothiazoline-4-carboxylic acid (ATCA) in biological samples, such as blood, urine, liver, and brain samples. The purpose of this study was to determine the optimal conditions for the quantification of ACTA in various biological fluids and organs using solid phase extraction (SPE) with a mixed-mode cation exchange cartridge, and high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Conditions associated with SPE sample loading, washing and final elution were studied and refined. The effect of ATCA signal fluctuations arising from electrospray ionization suppression was minimized by using a structural analogue of ATCA (2-aminothiazole-4-carboxylic acid, ATZA) as an internal standard. 0.5% trifluoroacetic acid in methanol was found to be a mobile phase that effectively eluted ATCA and ATZA. Symmetric peak shapes were obtained that were well suited to quantification. Mice received three different sublethal doses (6, 8, and 10 mg/kg) of KCN subcutaneously (3 mice / doses) and were terminated 15 mins after cyanide exposure. Exogenously elevated levels of ATCA were compared to endogenous levels of ATCA present in the biological fluids and organs. ATCA concentration in mice blood samples increased from 189 +/- 28 ng/mL to 413 +/- 66 ng/mL after a 10 mg/kg body weight dose of KCN was introduced subcutaneously. The endogenous ATCA concentrations found were as follows: 1.2 +/- 0.1 μg/g for kidney samples, 1.6 +/- 0.1 μg/g for brain samples, 1.8 +/- 0.2 μg/g for lung samples, 2.9 +/- 0.1 μg/g for heart samples, and 3.6 +/- 0.9 μg/g for liver samples. This method, which uses LC-MS/MS to detect ATCA as a biomarker for cyanide poisoning will be used in future toxicokinetic studies involving different biological matrices, and other animal species. The fundamental goal will be to gain a more complete picture of the toxicokinetics of cyanide poisoning.

PS 1690 MOLECULAR DOSIMETRY AND HALF LIFE OF N^2-HYDROXYMETHYL-DG ADDUCT IN RATS EXPOSED TO FORMALDEHYDE.

K. Lu[1], B. Moeller[2], M. Doyle-Eisele[3], J. McDonald[3] and J. A. Swenberg[1, 2]. [1]*Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC,* [2]*Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and* [3]*Lovelace Respiratory Research Institute, Albuquerque, NM.*

Formaldehyde is classified as a known human and animal carcinogen, causing nasopharyngeal cancer. Additionally, limited evidence for leukemia in humans is available; however, this is inconsistent across studies. Both genotoxicty and cytotoxicity are key events in formaldehyde nasal carcinogenicity in rats, but no mechanistic data exist for leukemia. In this study, a highly sensitive nano-LC-MS/MS-SRM method was developed and [$^{13}CD_2$]-formaldehyde exposures utilized, allowing differentiation of DNA adducts originating from endogenous and inhalation-derived formaldehyde exposure. Rats were exposed to 0.7, 2, 6, 10, 15 ppm [$^{13}CD_2$]-formaldehyde for 6h and N^2-hydroxymethyl-dG adducts in DNA from rat nasal epithelium were quantified by mass spectrometry. The number of exogenous N^2-HO$^{13}CD_2$-dG were 0.039±0.019, 0.19±0.08, 1.04±0.24, 2.02±0.44, 11.15±3.01 adducts/10^7 dG in rats exposed to 0.7, 2, 6, 10, 15 ppm [$^{13}CD_2$]-formaldehyde, respectively. Endogenous N^2-HOCH$_2$-dG in the nasal tissue was 4.70±1.80 adducts/10^7 dG. The results clearly show a nonlinear dose response, as demonstrated by the fact that 21 fold increase in exposure (0.7 to 15 ppm) induced 287 fold higher amounts of exogenous DNA adducts in nasal epithelium. In addition, we have performed a study to determine the half life of exogenous formaldehyde DNA adducts by exposing rats to 10 ppm [$^{13}CD_2$]-formaldehyde for 1 day (6h), followed by sacrificing animals at 6, 12, 24, 48 and 72 h, respectively. Our preliminary data indicate a rapid loss of nearly half of the adducts during the first 6 hours post exposure, followed by a constant loss over the rest of the 72 hour experiment with a half-life about 94 hours. This study provides strong data to support mode of action for inhaled formaldehyde in the target tissue for carcinogenesis and critical evidence for the science-based risk assessment of formaldehyde exposure.

PS 1691 FORMALDEHDYE-INDUCED HYDROXYMETHYL DNA ADDUCTS IN RATS EXPOSED TO ISOTOPE LABELED METHANOL.

H. Gul, K. Lu, P. Upton and J. A. Swenberg. *Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC.*

Formaldehdye is formed as an intermediate metabolite of methanol. As such, it could induce DNA damage following methanol exposure. However, it is difficult to measure the number of DNA adducts caused by methanol exposure since it is confounded by the presence of a substantial natural background formaldehyde adducts. In order to distinguish DNA adducts originating from endogenous formaldehdye and methanol-derived formaldehdye, we used highly sensitive mass spectrometry coupled with exposure to [$^{13}CD_4$]-methanol. Rats were exposed to 500 mg/kg/day and 2000 mg/kg/day by gavage for 5 days. Both N^2-hydroxymethyl-dG and N^6-hydroxymethyl-dA adducts were quantified by capilary LC-ESI-MS/MS. Endogenous N^6-hydroxymethyl-dA addcuts were present at 1~3 adducts/10^7 dA across different tissues, while endogenous N^2-hydroxymethyl-dG addduct were shown to be 3~6 adducts/10^7 dG. The number of [$^{13}CD_4$]-methanol derived exogenous dG adducts in the 500 mg/kg/day group was 0.08±0.08, 0.13±0.04, 0.12±0.04, 0.19±0.12, 0.37±0.08, 0.16±0.06, 0.09±0.03 adducts/10^7 dG for liver, lung, kidney, spleen, bone marrow, thymus and white blood cells, respectively. In the 2000 mg/kg/day dose group, the exogenous dG number was 0.41±0.14, 0.22±0.06, 0.39±0.09, 0.90±0.26, 1.42±0.29, 0.42±0.03 and 0.19±0.02 adducts/10^7 dG, respectively. In addition, [$^{13}CD_4$]-methanol administration caused a 1.3-2.3 fold increase in endogenous dG adducts. Interestingly, exogenous dA adducts were only detected in kidney (0.09±0.09 adduct/10^7 dA for 2000 mg/kg/day group) and bone marrow (0.10±0.08 and 0.29±0.09 adduct/10^7 dA for 500 mg/kg/day and 2000mg/kg/day group, respectively). This study provides the first unambiguous evidence that exogenous methaol-derived formaldehdye induced DNA damage is formed in multiple tissues. Our data also demonstrates that exogneous formaldehyde-dA adducts were formed in lower amounts in kidney and bone marrow. The reason for this tissue difference is under investigation.

PS 1692 HUMAN BIOMARKERS FOR TRICRESYL PHOSPHATE EXPOSURE.

J. Marsillach Lopez[1, 2], R. J. Richter[1, 2], T. B. Cole[1, 3], J. H. Kim[4], R. C. Stevens[1, 2], M. J. MacCoss[2], D. Tomazela[2], E. J. Hsieh[2], S. M. Suzuki[1, 2], L. M. Schopfer[5], O. Lockridge[5] and C. E. Furlong[1, 2]. [1]*Medicine, Division of Medical Genetics, University of Washington, Seattle, WA,* [2]*Genome Sciences, University of Washington, Seattle, WA,* [3]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA,* [4]*Anesthesiology, University of Washington, Seattle, WA and* [5]*University of Nebraska Medical Center, Omaha, NE.*

Tricresyl phosphate (TCP) caused thousands of cases of paralysis during Prohibition. TCPs are still used in jet-engine lubricants and hydraulic fluids. Air crew report tremors, memory loss and cognitive problems following cabin oil fume events. There is controversy whether these symptoms result from TCP exposure. ToCP isomers are metabolized by the liver into cresyl saligenin cyclic phosphate (CBDP), a neurotoxic metabolite that inhibits many serine active-site enzymes. Our aim was to identify biomarkers of exposure in blood samples from exposed individuals. We focused on plasma butyrylcholinesterase (BChE) and red cell acylpeptide hydrolase (APH). We developed single-step immunomagnetic bead-based protocols for purifying BChE and APH. CBDP-inhibited samples were used to identify and characterize the adducted active-site peptides. Samples were analyzed by high resolution mass spectrometry (MS). A peptide database search of the acquired data identified active site serine modifications, corresponding to +80 and +170. Using Selected Reaction Monitoring (SRM) we detected an increase in the abundance of the modified active site peptides in exposed samples compared to controls. We are using a chromatography alignment and feature detection software, CRAWDAD, to compare and detect differences across the samples. We expect that either the modification in the active site peptide in subjects is of very low abundance or the in vivo modifications are different from those observed in vitro. Supported by U.S. Army Medical Research & Materiel Command (W81XWH-07-2-0034), NIH (U01 NS058056, P30CA36727, R01ES09883, and P42ES04696), funding from pilot and flight attendant unions, Royal Australian Air Force, Norwegian Union of Energy Workers (SAFE), and NYCO SA.

PS 1693 VOC SERUM LEVELS IN THE GENERAL U.S. POPULATION: AN ANALYSIS OF THE 2003-2004 NHANES DATASET.

L. Fitzgerald, B. Burkhalter, J. Urban, D. Staskal, M. Harris and L. Haws. *ToxStrategies, Austin, TX.*

Serum levels of volatile organic compounds (VOCs) are increasingly being measured in people as part of exposure investigations designed to assess potential environmental exposures. Given the breadth of VOC sources, it is essential to have an understanding of typical background levels found in people. Data from the National Health and Nutrition Evaluation Survey (NHANES) provide information on serum levels of many compounds in the general U.S. population, so it is useful for characterizing background levels. As such, the objective of this analysis was to derive serum reference values for the U.S. population using the 2003-2004 NHANES dataset. Weighted summary statistics were developed for 21 VOCs commonly measured in community exposure investigations, and stratified by smoking status and other relevant demographic variables (gender, age and ethnicity). Of the 21 VOCs evaluated, 12 were found to be non-detect in all samples: 1,1,2-trichloroethane, 1,1-dichloroethane, 1,1-dichloroethene, 1,2-dibromo-3-chloropropane, 1,2—dichlorobenzene, 1,2-dichloroethane, 1,2-dichloropropane, 1,3-dichlorobenzene, chlorobenzene, cis-1,2-dichloroethene, hexachloroethane and trans-1,2-dichloroethene. Six of the remaining VOCs (1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, carbon tetrachloride, dibromomethane, methylene chloride and trichloroethene) had detection frequencies less than 2% and thus the mean and 95th percentile serum levels were very near or at the LOD/√2. Only 3 VOCs had detection frequencies greater than 15‰. The mean and 95th percentile concentrations, respectively, for these compounds were: 0.91 and 3.17 ng/ml for 1,4-dichlorobenzene, 0.04 and 0.12 ng/ml for styrene, and 0.08 and 0.14 ng/ml for tetrachloroethene. Only styrene had significantly elevated mean and median serum levels among smokers, regardless of smoking biomarker. The concentrations of other VOCs were not impacted by smoking status or demographics. These summary statistics provide a critical reference tool for assessing potential exposure to VOCs, as well as identifying potential populations of concern.

PS 1694 IDENTIFICATION OF URINARY METABOLIC PROFILES ASSOCIATED WITH METHYLMERCURY EXPOSURE USING A METABOLOMIC APPROACH.

P. Tremblay[1, 2], É. Gaudreau[2], É. Dewailly[1] and P. Ayotte[1, 2]. [1]*Axe de Recherche en Santé des Populations et Environnementale, CRCHUQ-CHUL and Université Laval, Quebec, QC, Canada and* [2]*Laboratoire de Toxicologie, Institut National de Sante Publique du Quebec, Quebec, QC, Canada.*

The metabolomic approach includes as a first step the quantitative measurement of the largest array possible of metabolites in biological matrices, while the second step consists of a multivariate statistical analysis to generate and identify metabolic profiles associated with the exposure under study. The purpose of the present study was to identify new urinary biomarkers associated with exposure to methylmercury (MeHg) through fish consumption in Cree adults from Northern Quebec (Canada). Separate analyses were performed in parallel for men (39 participants) and women (44 participants). For each sex, two groups were formed that were based on total blood mercury levels (high:> 70 nmol/L or low: <12 nmol/L). Additional groups of southern Quebec men and women were included for comparative purposes. The analysis of urine samples was performed by ultra performance liquid chromatography coupled with time of flight-mass spectrometry (UPLC-TOFMS). The MarkerlynxTM software (Waters) was used to identify metabolites (biomarkers) whose relative intensities differed between groups with high and low blood mercury levels. The multivariate analysis yielded several potential biomarkers and most of them were sex-dependent. We observed that differences in peak intensity between groups with high and low mercury levels were greater in men than in women for most potential biomarkers. Hence the metabolomic approach allowed the preliminary identification of several biomarkers related to MeHg exposure in this aboriginal population. Further analyses will be performed using other analytical platforms in order to generate additional potential biomarkers and attempt further identification of these unknown molecules.

PS 1695 QUANTIFICATION OF BENZO(A)PYRENE IN HAIR AS A BIOMARKER OF EXPOSURE USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY-SELECTIVE ION MONITORING (GC/MS-SI).

S. C. Campbell[1], D. E. Rollins[1], S. G. Tiffany[2] and D. G. Wilkins[1]. [1]*Pharmacology and Toxicology, University of Utah, Salt Lake City, UT and* [2]*Psychiatry, University of Buffalo, Buffalo, NY.*

Benzo(a)pyrene (B(a)P) is the most thoroughly studied polycyclic aromatic hydrocarbon (PAH) and is cytotoxic, genotoxic, mutagenic, and teratogenic in various tissues and cell types. Analysis of B(a)P in hair may provide a useful tool to monitor exposure in many different settings. We hypothesized that a sensitive GC/MS-SIM method could be developed to quantify B(a)P in hair from Long-Evans rats exposed to B(a)P. Prior to exposure, rats were pre-shaved in a specified region (baseline determination). Rats were then administered by i.p. either 40 mg/kg B(a)P in corn oil (n=10), or corn oil alone (n=5), once daily for seven days. Following an additional seven days to allow for re-growth, hair was again shaved in the specified region and stored in air tight containers in the dark at 4°C. On the day of analysis, 10 mg of hair was weighed and B(a)P-d12 internal standard added. Calibrators were concurrently prepared by fortification of B(a)P to analyte-free (blank) rat hair. After digestion of hair (sonication in 0.25 N NaOH for 2 hrs), samples were extracted three times with n-hexane and organic fractions dried under a constant stream of nitrogen. Residues were reconstituted in 1:1, acetone/dichloromethane and analyzed by our GC/MS-SIM method. The assay was determined to be linear from 0.25 to 100 ng/mg. Intra- and inter-day accuracy and precision were <25% CV for quality control samples fortified at 0.75, 40, and 80 ng/mg of hair. The measured concentrations of B(a)P in in the hair of the dosed rats ranged from 1.21- 2.35 ng/mg (mean: 1.55 + 0.32 SD). B(a)P was not detected in hair from control rats or baseline samples. The described method is simple, accurate, sensitive and specific, and will be used to support studies on the incorporation of B(a)P into hair and its use as a biomarker of exposure in heavy smokers and in children exposed to second hand cigarette smoke. Supported by NCI: R01 CA12040412.

PS 1696 BIOMARKERS OF CARDIOVASCULAR RISK IN NAVAJO POPULATIONS EXPOSED TO CONTAMINATED URANIUM MINING SITES.

M. Harmon[1], M. Channell[1], M. Campen[1], E. Erdei[2], M. Downs[2], B. Pacheco[2], D. Malony[3], M. Cajero[2], J. DeGroat[2], C. Shuey[4], S. Henio-Adeky[4], S. Ramone[4], T. Nez[4] and J. Lewis[2]. [1]*Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM,* [2]*Community Environmental Health Program, University of New Mexico, Albuquerque, NM,* [3]*Nephrology, University of Texas Houston Medical Center, Houston, TX and* [4]*Southwest Research and Information Center, Albuquerque, NM.*

Environmental exposure to heavy metal contaminants may promote or exacerbate cardiovascular disease. Numerous abandoned mines within the Navajo Nation contribute uranium, arsenic, radium and other heavy metals to the soil and groundwater. To assess the potential impact of these contaminants of cardiovascular health of exposed individuals, we have begun to examine traditional (IL-6, CRP) and novel (oxLDL, LOX-1) plasma biomarkers in a large cohort of residents of the Navajo Nation. Participants also provided self-report information on pre-existing conditions such as hypertension and diabetes. Samples and data were obtained through a culturally appropriate community-based participatory approach, incorporating data collection and outreach by Navajo community staff. Biomarker and self-report data were then linked to geospatial data on contamination sites using traditional linear regression and Bayesian models. Initial results from self-reported data indicate that proximity to contaminated sites is a significant predictor of hypertension, diabetes, and kidney disease incidence. Furthermore, in a small subsample (40 participants), oxLDL was significantly and positively associated with the proximity to contaminated sites by linear regression analysis (R=0.46; p=0.0029). Despite the limited sample size at present, these findings suggest that heavy metal contaminants from the legacy uranium mine tailings promote cardiovascular disease in affected populations. Future work will seek to link findings in novel biomarkers with more traditional clinical outcomes to provide valuable feedback to the communities at risk.

S 1697 DEVELOPMENTAL ORIGINS OF ADULT DISEASE: THE EFFECT OF LOW DOSE LEAD (PB).

E. P. Hines[1] and A. Rooney[2]. [1]*EMAG, NCEA, U.S. EPA, Research Triangle Park, NC and* [2]*National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction, NIEHS, Research Triangle Park, NC .*

Numerous epidemiology and animal toxicology studies support an association between an adverse prenatal environment and the development of diseases in later life. Experimentally, prenatal exposure to lead (Pb) at low, environmentally-relevant

doses causes physiological changes that increase the likelihood of diseases in adulthood such as obesity, hypertension, and neurological disorders. Although human epidemiological data also support an association between increased Pb exposure and hypertension or cognitive impairment, the exact mechanisms by which lead exerts these effects in epidemiologic studies is unknown. We will discuss experimental animal toxicology data that suggest several possible mechanisms for developmental origins of adult diseases (DOAD) associated with exposure to low levels of lead. Data support a sex-dependent mechanism resulting in increased fat deposition and late-onset obesity, retinal degeneration, and motor activity aberrations following low dose gestational lead exposure in male mice. Lead-induced hypothalamic pituitary adrenal axis dysfunction is a potential mechanism for a range of adult diseases and disorders, including hypertension, diabetes, metabolic syndrome, schizophrenia, and cognitive dysfunction. Epigenetic mechanisms have also been implicated, and epigenetic pathways may present a mechanistic link between developmental lead exposure and the etiology of Alzheimer's disease. These data highlight the importance of exposure windows for the development of adverse health effects associated with low-level lead and have possible risk assessment implications for ongoing assessments such as the NTP Monograph on Low-Level Lead and the U.S. EPA Integrated Science Assessment for Lead.

S 1698 LOW-LEVEL GESTATIONAL LEAD EXPOSURE IS A RISK FACTOR FOR LATE-ONSET METABOLIC SYNDROME AND NEURODEGENERATION.

D. A. Fox. *University of Houston, Houston, TX.*

Obesity is a pandemic as 65% of adolescents and adults are overweight and 30% are obese. Epidemiological studies show a positive association between developmental lead exposure and increased body mass index (BMI) during adulthood. Increased BMI also shows a significant inverse log-linear relationship with blood [Pb] ([BPb]). There are several critical periods of nervous and immune system growth and development during which toxicant exposure can increase susceptibility to diseases later in life. To investigate the effects of low-level gestational lead exposure (GLE) on late-onset metabolic and neurodegenerative, we developed a human equivalent gestational lead exposure (GLE) model. Mice were exposed to three different levels of lead during pregnancy until postnatal day 10 (PN10): period of rodent brain/retinal development equivalent to that during human gestation. Peak [BPb] for controls, low-, moderate- and high-level lead groups on PN10 were <1, ~10, ~25 and ~40 μg/dL, respectively, and on PN30 all GLE [BPb] were not different from controls. GLE produced no differences in pup or dam weights, dam food-fluid intake during GLE, or food-fluid consumption in 9-15 month male or female offspring. At 12 and 15 months, relative to controls, GLE males had increased body weight; abdominal, gonadal and subcutaneous fat weights; fasting blood glucose levels; and plasma TNFα levels. The effects were nonmonotonic and characterized by an inverted U-shaped curve. Furthermore, the aged GLE male mice had enhanced late-onset retinal degeneration compared to age-matched control mice. Aged female GLE mice had no differences on any of these measures, relative to controls. These results show that GLE-induced obesity in aging males was not due to increased food intake. GLE produced altered body composition, hyperglycemia, systemic para-inflammation and enhanced retinal degeneration in aged males. In summary, these results suggest that low-level GLE is a long-term risk factor for obesity-related metabolic syndrome and possibly neurodegenerative diseases: especially in males. Supported by NIH Grants ES012482, EY07551 and EY07024.

S 1699 DEVELOPMENTAL LEAD (PB) EXPOSURE AND PERMANENT HPA AXIS DYSFUNCTION: A POTENTIAL UNIFYING BIOLOGICAL MECHANISM FOR PB-ASSOCIATED DISEASES AND DISORDERS.

D. A. Cory-Slechta. *Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY.*

Hypothalamic-pituitary-adrenal (HPA) axis dysfunction is associated with hypertension, diabetes, metabolic syndrome, schizophrenia, and cognitive dysfunction, many of which have also been associated with elevated lead (Pb) levels. These diseases, like elevated blood Pb levels, exhibit a higher prevalence in low socioeconomic status communities, a phenomenon hypothesized to reflect elevated stress levels in such environments. Thus, elevated Pb and stress are co-occurring risk factors. They also share biological substrates, raising the possibility that combined Pb exposure and stress may produce enhanced adverse effects. Our animal models show that both maternal only and lifetime Pb exposures, at blood Pb values at or below the CDC's level of concern, can permanently alter HPA axis function, and do so differentially by gender. Such changes include dynamic alterations in corticosterone levels, nuclear and cytosolic glucocorticoid receptor levels, glucocorticoid negative feedback and responsiveness to stress challenges. Further, Pb exposure combined with prenatal stress (PS), or PS followed by offspring stress (OS), can produce enhanced toxicity manifest in behavior, regional neurotransmitter levels, and brain cytokine profiles. Enhanced behavioral impairments arising from combined Pb and stress have included context-dependent learning deficits in females and increased rates of responding on Fixed Interval schedules of reinforcement, a behavior deemed a surrogate for impulsivity in children. Inter-correlations among these behavioral alterations, regional catecholamine changes and corticosterone levels suggest HPA axis alteration-induced neurochemical changes as the basis of behavioral toxicity. Thus, developmental Pb exposure, by permanently altering HPA axis function, may contribute to lifelong CNS alterations, effects that can be further enhanced in conjunction with PS or OS. These findings underscore the critical importance of studying toxicants such as Pb in the context of other pertinent risk factors.

S 1700 EARLY LIFE EXPOSURE TO PB AND PROGRAMMED SUSCEPTIBILITY TO NEURODEGENERATIVE DISEASE.

N. Zawia. *Biomedical Sciences, University of Rhode Island, Kingston, RI.*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose clinical manifestations appear in old age. The majority of AD cases occur in the elderly; however, it is still unresolved whether AD is a disease of old age or whether it has earlier beginnings. The development of early onset AD (EOAD) seems to be largely genetic, however, late-onset AD (LOAD), a common sporadic form of the illness, maybe influenced by epigenetic factors acquired during early developmental stages. Accumulating evidence suggests that LOAD may result from the combined effects of variation in a number of genes and environmental factors. In comparison to monogenic diseases, LOAD exhibits numerous non-Mendelian anomalies that suggest an epigenetic component in disease etiology. The sporadic nature of >90% of AD cases, the differential susceptibility and course of illness, as well as the late age onset of the disease suggests that epigenetic and environmental components play a role in the etiology of LOAD. One of the first indications that epigenetics was involved in LOAD came from studies from our lab (Basha et al., 2005; Wu et al., 2008; Zawia et al., 2009) which showed that lead (Pb) exposure occurring during brain development pre-determined the expression and regulation of AD-related genes later in life, influencing the course of amyloidogenesis and oxidative DNA damage via a process that involved DNA methylation. This presentation will focus on the mechanisms by which early life exposure to Pb can modify DNA through epigenetic pathways rendering DNA repair pathways ineffective in old age to counter oxidative damage. The interaction between DNA methylation and DNA oxidation and their relevance to Alzheimer's disease will be discussed. In addition, a global assessment of gene expression and DNA methylation across the lifespan of developmentally exposed animals will be presented. The integration of global gene expression profiles, with simultaneous consideration of both genetic and epigenetic characteristics and of the interactions between these factors and aging, will shed some light on the complex pathobiology of neurodegeneration.

S 1701 CONTRASTING THE DEVELOPMENTAL AND ADULT ORIGINS OF ADVERSE EFFECTS FROM LEAD IN THE DRAFT NTP MONOGRAPH ON LOW-LEVEL LEAD.

A. A. Rooney. *Center for the Evaluation of Risks to Human Reproduction, NIEHS, National Toxicology Program, Research Triangle Park, NC.*

Epidemiological evidence supports a broad range of effects associated with elevated blood lead (Pb) levels including neurological, cardiovascular, renal, immune, reproductive and developmental effects. Children are regarded as particularly vulnerable to Pb due to exposure issues leading to higher blood Pb levels in children as well as data suggesting that children are more sensitive to some of the health effects associated with Pb exposure. In particular, numerous studies report impaired cognitive function associated with developmental or childhood exposure to Pb at blood lead levels < 10 micrograms/dL, whereas effects on cognitive function in adults are generally associated with higher blood Pb levels. Immunological studies also suggest that early development may be a period of greater susceptibility to Pb as several epidemiological studies report an association between developmental exposures to Pb at low levels with elevated serum IgE. The increased production of IgE suggests a mechanism by which Pb may contribute to the development of allergic asthma and later life allergic disease. For other endpoints, such as reduced renal function, increased blood pressure and hypertension, the deleterious effects associated with low blood Pb levels in adults are well established; however, fewer studies address the impact of developmental exposure. The NTP Center for the Evaluation of Risks to Human Reproduction is developing an NTP Monograph on Low-Level Pb that will evaluate the scientific evidence regarding the potential health effects associated with low exposure levels of lead (i.e., blood Pb levels < 10 micrograms/dL). The examples listed above illustrate the importance of exposure timing for the development of some Pb-related adverse health effects, a factor that will be considered in the NTP's evaluation of low-level Pb.

S 1702 DOES THE CLOCK MAKE THE POISON? INFLUENCE OF THE CIRCADIAN CLOCK ON TOXICOLOGICAL MECHANISMS AND OUTCOMES.

H. Zarbl[1] and L. A. Hooven[2]. [1]*Environmental and Occupational Health Sciences Institiute, Robert Wood Johnson Medical School, Piscataway, NJ and* [2]*Department of Zoology, Oregon State University, Portland, OR.*

The daily biochemical, physiological, and behavioral activities of many organisms are synchronized by light/dark cycles. These temporal oscillations are maintained by the circadian clock, which has intrinsic periodicity of approximately 24 hours. The circadian rhythm of cells is controlled by the interaction of multiple positive and negative feedback loops comprising a molecular oscillator, which modulates expression through transcriptional and epigenetic means. Environmental and occupational exposures leading to disruption of circadian rhythm, including jet lag, light-at-night, and shift work are associated with an increased risk of endometriosis, breast cancer, and prostate cancer, prompting the International Agency for Cancer Research to classify shift work as a probable human carcinogen (type 2A). Disruption of the circadian clock is also associated with increased risk of cancer, cardiovascular disease, diabetes, obesity, reproductive problems, sleep disorders, drug and alcohol addiction, and psychiatric disorders. Accumulating evidence indicates that the dynamic influence of the circadian clock profoundly affects many critical pathways in toxicology. Exposure to stressors, carcinogens, chemotherapeutic agents, and other xenobiotics can alter circadian function in cells, rodents, and humans, while some chemopreventive agents my reset the rhythm. Targeted disruption of clock gene expression is advancing our ability to understand and manipulate the circadian clock. Our panel of experts will review the state of this emerging area and explore opportunities for disease prevention.

S 1703 ENVIRONMENTAL INFLUENCES UNCOUPLE PERIPHERAL CLOCKS FROM SCN CLOCKS.

U. Schibler. *Molecular Biology, University of Geneva, Geneva, Switzerland.* Sponsor: H. Zarbl.

Cell-autonomous and self-sustained circadian oscillators are operative in virtually all body cells of mammalian organisms. These remarkably robust clocks must be synchronized periodically by a master circadian pacemaker residing in the suprachiasmatic nucleus (SCN) of the brain to keep phase coherence. This is accomplished through a variety of signaling pathways employing blood-borne factors (e.g. cyclically secreted hormones and growth factors), metabolites, body temperature, and neuronal cues. I shall present experimental strategies for the identification of signaling pathways that participate in the synchronization by feeding cycles, oscillating blood-borne signals, and body temperature rhythms. NAD+-sensing enzymes, such as Sirtuin deacetylases and poly(ADP-ribose)polymerase 1 (PARP-1) are likely candidates for conveying the metabolic state of a cell to its circadian oscillator. We demonstrated that both SirT1 and PARP-1 indeed impart on circadian gene expression, and that PARP-1 participates in the feeding-dependent synchronization of liver circadian clocks. A novel technology, dubbed STAR-PROM (for Synthetic Tandem Repeat Promoter, to screen for all immediate early transcription factors (IETFs) that serve as sensors of blood-borne signals revealed 14 artificial promoters containing binding sites for IETFs that respond differentially to human and/or rat plasma samples collected at three- to four-hour time intervals around the clock. These IETFs are candidates for being components of signaling pathways that are stimulated by cyclic systemic blood-borne signals regulated directly or indirectly by the circadian master pacemaker in the SCN. Simulated mouse temperature rhythms with an amplitude of only 8,5% (min. 35 °C, max. 38 °C) are also capable of synchronizing the clocks of cultured fibroblasts. Using MEFs from the relevant knockout mice and by performing RNA interference in NIH3T3 cells we demonstrated that both HSF1 and the cold-inducible RNA binding protein CIRP are required for the efficient phase entrainment by body temperature rhythms.

S 1704 TICK-TOX: CLOCK GENE EXPRESSION AND INTERACTIONS BETWEEN THE MOLECULAR PATHWAYS FOR THE REGULATION OF CIRCADIAN RHYTHMS AND TOXIN METABOLISM.

D. J. Earnest. *Neuroscience and Experimental Therapeutics, Texas A&M Health Sciences Center, College Station, TX.* Sponsor: H. Zarbl.

Members of the Per-Arnt-Sim (PAS) family of transcriptional regulators are involved in development and in sensing and adapting to environmental changes. Most PAS proteins function as heterodimers consisting of a sensor protein complexed with a general binding partner and through these interactions mediate biological responses to environmental conditions. In this regard, the PAS protein, aryl hydrocarbon receptor (AhR) partners with AhR nuclear translocator (ARNT) to mediate the metabolism of drugs and environmental toxins. Because AhR and ARNT are commonly expressed in the cells and tissues throughout the body with other PAS genes in molecular feedback loops forming the mammalian circadian clockworks, it is possible that functional interactions between these PAS protein-regulated pathways may have implications for the biological consequences of xenobiotic exposure. To examine the role of key PAS proteins in the circadian clockworks in modulating xenobiotic metabolism, we determined whether the toxin-mediated activation of the AhR signaling pathway in the mammary gland and liver: 1) fluctuates rhythmically; and 2) is altered following targeted disruption or siRNA inhibition of key PAS genes in the circadian clockworks, Period 1 (Per1) and Per2. In both the mouse mammary gland and liver, the inductive effects of the prototypical Ahr ligand, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on a key target of AhR signaling, Cyp1A1, were marked by diurnal variation such that TCDD-induced Cyp1A1 expression was 23-43 fold greater during the night than during the daytime. In mutant mice, targeted disruption of Per1 alone or Per1 and Per2 (Per1ldc and Per1ldc/Per2ldc) abolished this diurnal rhythm in the toxin-mediated induction of hepatic and mammary gland Cyp1A1 expression and significantly increased the TCDD-induced expression of p450 genes. These studies indicate that the clock genes, Per1 and Per2, may function as inhibitory factors in the diurnal modulation of AhR-regulated responses to toxins and thus have important implications for intercommunication between these PAS protein-regulated pathways.

S 1705 THE HEPATOCYTE AUTONOMOUS CLOCK MODULATES THE CHRONOTOXICITY OF ACETAMINOPHEN.

C. A. Bradfield[1], J. A. Walisser[1], B. P. Johnson[1], Y. Liu[1], A. Shen[1], E. L. McDearmon[1], B. McIntosh[1], A. Vollrath[1], A. C. Schook[2] and J. S. Takahashi[2]. [1]*The McArdle Laboratory for Cancer Research, University of Wisconsin School of Medicine and Public Health, Madison, WI and* [2]*Northwestern Univeristy, Evanston, IL.*

The hepatotoxicity of Acetaminophen (APAP) was first reported in the 1960s and its circadian changes in metabolism in the 1970s. The dose independent circadian variation in APAP hepatotoxicity is thought to be primarily due to oscillations of liver GSH and possibly changes in cytochrome p450 enzymatic activity which follow eating patterns. The circadian clock drives the 24 hour oscillations in these factors directly and indirectly (e.g. by direct regulation of drug metabolizing enzymes or by driving feeding rhythms). Because the circadian clocks of various tissues can become uncoupled or disrupted under conditions such as shift work, we set out to determine the relative contributions of the central clock in the suprachiasmatic nucleus (SCN) and the hepatocyte circadian clock in modulating the chronotoxicity of APAP. Using mice with conditional null alleles of the Mop3 locus, we were able to generate livers harboring hepatocytes with little or no cell autonomous circadian rhythms. The observation that these mice become resistant to the chronotoxicity of APAP suggests that while the central clock modulates detoxification indirectly through feeding rhythms and GSH; the hepatocyte clock controls daily oscillations in major aspects of APAP bioactivation by the cytochrome P450 system.

S 1706 CIRCADIAN EXPRESSION OF DRUG PROCESSING GENES IN MICE.

C. D. Klaassen. *Pharmacology, University of Kansas Medical Center, Kansas City, KS.*

Temporal coordination of hepatic drug-processing gene (DPG) expression facilitates absorption, biotransformation, and excretion of exogenous and endogenous compounds. To further elucidate the circadian rhythm of hepatic DPG expression, mice were subjected to a standard 12-h light/dark cycle, and livers were collected six times a day. The mRNAs of hepatic phase I enzymes (cytochromes P450, aldehyde dehydrogenases, and carboxylesterases), phase II enzymes (glucuronosyltransferases, sulfotransferases, and glutathione-S-transferases), uptake and efflux transporters, and transcription factors were quantified. In general, the mRNA of phase I enzymes increased during the dark phase, whereas the mRNAs of most phase II enzymes and transporters reached maximal levels during the light phase. The majority of hepatic transcription factors exhibited expression peaks either before or after the onset of the dark phase. During the same time period, the negative clock regulator gene Rev-Erbα and the hepatic clock-controlled gene Dbp also reached mRNA expression peaks. Considering their important role in xenobiotic metabolism, hepatic transcription factors, such as constitutive androstane receptor, pregnane X receptor, aryl hydrocarbon receptor, and peroxisomal proliferator activated receptor α, may be involved in coupling the hepatic circadian clock to environmental cues. Taken together, these data demonstrate that the circadian expression of the DPG battery and transcription factors contribute to the temporal detoxification cycle in the liver.

S 1707 RELEVANCE OF CIRCADIAN DISRUPTION AND CIRCADIAN INDUCTION FOR CANCER CONTROL.

F. Levi. *Rhythmes Biologiques et Cancer, INSERM, Villejuif, France.* Sponsor: H. Zarbl.

The circadian timing system drives daily changes in xenobiotic metabolism and detoxification, cell cycle events, DNA repair, apoptosis, and angiogenesis. As a result the main hallmarks of cancer, including inflammation, are under circadian control. Cancer promotion and/or progression are accelerated in rodents with circadian disruption resulting from ablation of the suprachiasmatic nuclei, the central pacemaker; chronic jet lag; or clock gene mutation. Circadian disruption is also involved in chemical carcinogenesis. Cancer patients with circadian disruption display poor survival, as compared with those with robust circadian rhythms, despite similar clinical characteristics. Clock genes are down-regulated in most experimental and human cancers and impairment of tumor molecular clocks can accelerate cancer growth. Circadian induction in tumor tissue with defective circadian clocks using timed seliciclib or meals slows tumor growth in mice. Meal timing induces the rhythmic expression of over 400 genes for tumor growth in a pancreatic tumor. We show that the circadian amplitude of core body temperature is critical for circadian induction in tumor and implicates cell stress signaling pathways in tumor transcriptome entrainment. Circadian timing can also modify the tolerability and efficacy of anticancer drugs in patients. Improved efficacy correlates with the respective times of best tolerability, due to (a) inherently poor circadian entrainment of tumors and (b) persistent circadian entrainment of healthy tissues. Conversely, host clocks are disrupted whenever anticancer drugs are administered at their most toxic time, while speeding cancer processes. Mathematical and systems biology approaches currently develop and integrate theoretical, experimental, and technological tools in order to further optimize and personalize the circadian administration of cancer treatments. The detection of early warning signals (temperature and rest-activity) may provide relevant decision making information for the personalization of cancer chronotherapeutics.

S 1708 METHYLSELENOCYSTEINE RESETS THE RHYTHMIC EXPRESSION OF CIRCADIAN AND GROWTH REGULATORY GENES DISRUPTED BY NITROSOMETHYLUREA *IN VIVO.*

M. Fang and H. Zarbl. *Environmental and Occupational Health Sciences Institiute, Robert Wood Johnson Medical School, Piscataway, NJ.*

The role of Selenium (Se) in chemoprevention of mammary carcinomas was first suggested by animal studies performed by Ip and coworkers who showed that feeding carcinogen-treated rats garlic grown in selenium enriched soil, dramatic reduced tumor incidence. Subsequent studies demonstrated that methylselenocysteine (MSC), the active form of Se, mediates its effects at the post-initiation stage of carcinogenesis. However, the in vivo mechanisms of MSC-mediated chemoprevention are still poorly understood at the molecular level. To investigate how a dietary MSC is able to reduce the incidence of mammary carcinoma in pubescent female Fischer 344 (F344) rats exposed to N'-nitoso-N'-methyurea (NMU), we used DNA microarrays and qPCR to compare gene expression profiles in mammary tissue of NMU-treated F344 rats fed a MSC-enriched diet (3 ppm Se) to that of rats on a standardized diet (0.1 ppm Se). Unexpectedly, the most dramatic effect of dietary MSC supplementation was on the genes involved in the network of circadian rhythm, providing the first evidence for the link between circadian rhythm and chemoprevention. Exposure to a single carcinogenic dose of the alkylating agent, NMU, resulted in a persistent disruption in the rhythmic expression of several circadian genes, including Period 2 (Per2), Rev-ErbA α, the Melatonin Receptor 1α (MTNR1A), as well as estrogen receptor beta (ERβ), in contrast, dietary MSC significantly restored or enhanced the circadian expression of these genes during the early stage of mammary tumoregenesis. At meantime, dietary MSC also promotes circadian expression of genes essential to normal mammary cell growth and differentiation (ER[l#61472#l]b, Trp53, p21, Gadd45[l#61537#l]), suggesting an important role of enhanced-circadian rhythm on inhibition of tumoregenesis. Analysis of tumors that arising in animals fed a MSC-enriched diet showed that these tumors lost MSC-induced expression of circadian genes compared to normal mammary glands, indicating that circadian gene expression was incompatible with NMU-induced mammary carcinogenesis.

S 1709 MACROPHAGES: REGULATORS OF TOXICITY AND DISEASE PATHOGENESIS.

D. Laskin and A. Gow. *Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

Macrophages function as control switches of the immune system, providing a balance between pro- and anti-inflammatory responses. To accomplish this, they develop into different subsets: classically (M1) or alternatively (M2) activated macrophages. Whereas M1 macrophages display a cytotoxic, proinflammatory phenotype, M2 macrophages, suppress immune and inflammatory responses and participate in wound repair and angiogenesis. Critical to the actions of these divergent or polarized macrophage subpopulations is the regulated release of inflammatory mediators. When properly controlled, classically activated M1 macrophages effectively destroy invading pathogens, tumor cells, and foreign materials. However, when M1 activation becomes uncontrolled, these cells release excessive quantities of cytotoxic mediators that contribute to disease pathogenesis. The activity of M1 macrophages is countered by alternatively activated M2 macrophages which release mediators that down regulate M1 cells, and stimulate growth, extracellular matrix turnover, and tissue repair. Aberrant functioning of M2 macrophages can lead to fibrosis and tumor metastasis and progression. Ultimately, it is the balance in the production of mediators by these two cell types that determines the outcome of the tissue response to chemical toxicants and disease progression. These different models will be presented to illustrate this divergent role of macrophages in disease pathogenesis and toxicity.

S 1710 MACROPHAGES AND HEPATOTOXICITY: A BATTLE OF FORCES.

D. Laskin[1], C. Gardner[1], Y. Liu[1] and J. Laskin[2]. [1]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ and* [2]*UMDNJ-RWJ Medical School, Piscataway, NJ.*

Accumulating evidence suggests that macrophages are key in acetaminophen (APAP) hepatotoxicity. However, their role in the pathogenic process depends on the timing of their appearance in the liver, and the inflammatory mediators they encounter which direct their functional responses. Initially, macrophages responding to stimuli such as LPS, HMGB1 and/or TNFα release proinflammatory cytokines and cytotoxic mediators contributing to toxicity. Later in the pathogenic process, following exposure to IL-10 or IL-4/IL-13, they function to down regulate inflammation and initiate wound repair. These activities appear to be mediated by distinct subpopulations of macrophages exhibiting a classically (M1) or alternatively (M2) activated phenotype. This is supported by findings that APAP intoxication results in early accumulation of macrophages in the liver expressing M1 markers (e.g., TNFα, inducible nitric oxide synthase) followed by cells expressing M2 markers (e.g., arginase, galectin). Additionally, the appearance of M1 and M2 macrophage subpopulations correlates with evidence of oxidative stress and liver injury (reduced GSH, elevated serum transaminases) and tissue repair (hepatocyte proliferation, expression of the growth factors, CTGF and TGFβ), respectively. It appears that the switch in macrophage phenotype from M1 to M2 is a consequence of a changing cytokine environment. This is consistent with findings that the M2 chemokines and macrophage activators, MCP-1/CCR-2, IL-10 and IL-4/IL-13, are upregulated in the liver during the initial stages of APAP-induced tissue repair. Whereas pretreatment of animals with gadolinium chloride, an M1 inhibitor, protects against APAP induced hepatotoxicity, clodronate liposomes, which suppress M2 cells, exacerbates tissue injury. These data demonstrate that macrophages with distinct phenotypes play different roles in APAP hepatotoxicity; moreover the outcome of the response depends on the balance between these cell populations (NIH GM034310, ES005022 AR055073, ES004738, CA132624).

S 1711 LUNG MACROPHAGE RESPONSES TO BIOACTIVE ENGINEERED NANOMATERIALS (ENM) INVOLVES ACTIVATION OF THE NLRP3 INFLAMMASOME.

A. Holian and C. Migliaccio. *Center for Environmental Health Sciences, University of Montana, Missoula, MT.*

Exposure to ENM can lead to lung inflammation and fibrosis. The mechanisms underlying the development of these pathologies, and the characteristics that separate bioactive from benign ENM have not been established. Evidence suggests that cells of the innate immune system initiate and sustain chronic inflammation required for the development of pulmonary fibrosis. Alveolar and interstitial macrophages are the primary innate immune cells in the lung; subpopulations of these cells have been implicated in the development of chronic inflammation and fibrosis. A key pathway in the macrophage initiation of inflammation is the NLRP3 inflammasome. Activation of the NLRP3 inflammasome results in activated caspase-1 and cleavage of the pro-IL-1β family of cytokines into bioactive forms. Excessive and sustained productions of IL-1β, and possibly IL-18, appear to be responsible for chronic inflammation. Utilizing C57Bl/6 and Balb/c strains of mice, we evaluated the responses of a series of well-characterized multi-walled carbon nanotubes and titanium dioxide ENM by using in vitro (isolated AM) and in vivo systems. Our studies demonstrate that bioactive (causing lung inflammation and pathology) ENM consistently induce activation of the NLRP3 inflammasome by classically activated macrophages. In contrast, benign ENM cause little or no activation of the NLRP3 inflammasome. The results indicate that inflammasome activation can be predicted by shape and composition. Thus, long durable ENM and

those with high nickel content are significant activators of the inflammasome. The proposed mechanism involves uptake of the particles into lysosomes followed by lysosomal rupture and release of cathepsin B. Active cathepsin B stimulates the assembly of the NLRP3 inflammasome. Furthermore, a shift in macrophages towards a distinct subset, potentially the Th2-associated alternatively activated phenotype, may play a role in determining if ENM induces fibrosis. Supported by NIH ES015497, ES018742, RR017670 and NSP CBET0834233.

S 1712 MACROPHAGE DIVERSITY AND POLARIZATION IN IMMUNOPATHOLOGY.

A. Mantovani. *Istituto Clinico Humanitas IRCCS, University of Milan, Milan, Italy.* Sponsor: D. Laskin.

Plasticity is a hallmark of cells of the myelomonocytic lineage. Mononuclear phagocytes in response to innate recognition or to signals from lymphocytes undergo adaptive responses. Shaping of monocyte-macrophage function is a key component of resistance to pathogens, tissue damage and repair. Neutrophils have now revealed considerable plasticity, reminiscent of their macrophage 'cousins'. Orchestration of myelomonocytic cell function is a key element linking inflammation and cancer and provides a paradigm for macrophage plasticity and function. Better understanding of the molecular basis of myelomonocytic cell plasticity opens new vistas in immunopathology and therapeutic intervention. Thus, different macrophage subsets, which differentially contribute to plaque infiltration and to atherosclerosis complications, have been identified. Similarly, depending on different environmental signals, plaque-associated macrophages can express polarized pro- and anti-atherogenic programs by influencing lipid metabolism, inflammatory responses, and plaque stability. Thus, a "macrophage balance" plays a major role in the pathogenesis of atherosclerotic plaques and affects the evolution and complications of atherosclerosis.

S 1713 MECHANISMS OF MICROGLIAL ACTIVATION IN RESPONSE TO TOXICANTS.

A. Gow. *Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.* Sponsor: D. Laskin.

Macrophages are known to adopt an activated phenotype when challenged with pathogens or toxicants. Up-regulation of inducible nitric oxide synthase (iNOS) is a fundamental characteristic of classically activated macrophages. However the biological targets of iNOS-derived nitric oxide (NO) have not been fully elucidated and hence the role of NO in the macrophage activation process unknown. We recently identified a number of NO-related mechanisms for controlling macrophage activation, particularly with regard to the resulting phenotpye, vis a vis classical (M1) and alternative (M2) activation. The pulmonary collectin, surfactant protein-D (SP-D), serves to regulate the macrophage phenotype in response to LPS. NO-mediated modification of SP-D can alter this function to favor M1 or M2 depending upon its redox milieu. In this way iNOS-derived NO is both a regulator and potential product of activation. A key factor in this NO-mediated regulation is the redox state of the NO. Modification of SP-D by lower oxide NO leads to the generation of S-nitrosylated or SNO-SP-D, while higher oxides lead to cross-linked SP-D. SNO-SP-D favors M1 activation while cross-linked SP-D favors M2 activation. Monocyte-derived microglial cells in the brain also up-regulate iNOS expression in response to inflammatory activators, such as LPS. We found that NO-derived from iNOS is critical to the adoption of a M1 phenotype in microglial cells. Additionally, redox status appears to be key in this process. Redox active copper, (Copper I) inhibits the release of NO by LPS-treated microglial cells. This is independent of reduced iNOS expression or NF-κB activity. Immunofluorescence analysis macrophage activation markers have established that Copper (I) induces M2 type activation. As microglial activation is a critical step in neurodegeneration, these interactions may have implications for the role of environmental factors in aging-related neurological disease. Supported by NIH HL086621.

S 1714 MACROPHAGE DIVERSITY PROMOTES TUMOR PROGRESSION AND METASTASIS.

J. W. Pollard. *Department of Developmental and Molecular Biology, Albert Einstein College of Medicine Yeshiva University, New York, NY.* Sponsor: D. Laskin.

There is persuasive clinical and experimental evidence that macrophages promote cancer initiation and malignant progression. During tumor initiation, they create an inflammatory environment that is mutagenic and promotes growth. As tumors progress to malignancy, macrophages stimulate angiogenesis, enhance tumor cell migration and invasion, and suppress antitumor immunity. At metastatic sites, macrophages prepare the target tissue for arrival of tumor cells, and then a different subpopulation of macrophages promotes tumor cell extravasation, survival, and subsequent growth. Specialized subpopulations of macrophages with distinct phenotypes may represent important new therapeutic targets. Supported by NIH grant CA131270.

S 1715 WHEN IS EXPOSURE NOT EXPOSURE? DEFINING THE DOSE-RESPONSE REGION BETWEEN "EFFECT" AND "ADVERSE EFFECT" IMPLICATIONS FOR HUMAN HEALTH RISK ASSESSMENT.

C. English. *NSF International, Ann Arbor, MI.*

'Omics technologies demonstrate global changes in gene regulation and expression following chemical exposure, causing toxicologists to revisit the question, what is an adverse effect and what isn't. Dose-dependent transitions in genomic and related responses reflect the levels of exposure that cause detectable perturbations to the biological system under study. We will focus on two transitions, having both dose and temporal dimensions, which are advanced as being pivotal to understanding mode of action and prediction of toxicity. The first transition is from no-detectable-effect on the biological system relative to unexposed controls, to the first-perceptible-effect at the global genome level; observed as up- and down-regulation of genes that regulate adaptive responses. The second transition is from the adaptive response region to the first adverse response or critical effect region of the dose-response relationship. We will explore if and how 'omic phenomena elicited by chemical exposures translate into useful information for risk assessors. Consideration of the adaptive capacity of the biological system and severity of the effect might further inform our definition of the term adverse and inform the magnitude of traditional uncertainty factors used. Understanding dose-dependent transitions combined with dosimetry models that characterize the exposure-tissue concentration relationship might permit risk assessors to define exposures delimited by safe and adverse boundaries. We will conclude by describing emerging advances in high-throughput quantitative 'omic technologies, and findings from studies with endogenous and exogenous compounds and nanoparticulates, to address how we move from the vast array of 'omic data generated to practical risk assessment applications.

S 1716 USE OF THE HIERARCHICAL OXIDATIVE STRESS PARADIGM FOR HAZARD AND RISK ASSESSMENT IN RESPONSE TO AMBIENT ULTRAFINE AND ENGINEERED NANOPARTICLE TOXICITY.

A. E. Nel. *Medicine, University of California Los Angeles, Los Angeles, CA.* Sponsor: A. Nel.

The hierarchical oxidative stress paradigm describes the cellular or tissue response to incremental levels of oxidative stress in response to pro-oxidative ambient ultrafine (UFP) and engineered nanoparticles (ENM). The paradigm is useful for elucidating dose-dependent oxidative stress outcomes in which the critical effect could manifest as Nrf2-mediated antioxidant defense or pro-inflammatory signal pathway responses that vary with the particle oxidant potential and the level of redox disequilibrium. Clinically, this could manifests as adaptive or pro-inflammatory responses in the lung that can be detected by oxidative stress biomarkers. I will discuss the utility of the hierarchical oxidative stress paradigm in in vitro and in vivo studies looking at UFP and nano-ZnO effects. I will show how conventional and rapid throughput in vitro cellular screening can be used to demonstrate the hierarchical oxidative stress response to these particles and how the cellular response relates to pulmonary inflammation in rodents. I will demonstrate that Nrf 2 knockout exacerbates airway inflammation and that changing of the dissolution characteristics of nano-ZnO can affect the exposure dose and the severity of lung inflammation. In summary, the critical effect to pro-oxidative nanoparticles could be a useful screening tool for hazard ranking and risk prevention.

S 1717 HUMAN EXPOSURE CONTEXT FOR NANOPARTICLE TOXICITY ASSESSMENT BY BIOLOGICAL PATHWAY BASED DOSE-RESPONSE MODELING.

J. G. Teeguarden[1], M. N. Costa[2], K. M. Waters[2] and J. E. McDermott[2]. [1]*Biological Monitoring and Modeling, Pacific Northwest National Laboratory, Richland, WA and* [2]*Computational Biology and Bioinformatics, Pacific Northwest National Laboratory, Richland, WA.*

Dose-response modeling of in vitro gene expression profiles hold great promise for differentiating between mechanisms of action and between low-dose adaptive effects and more serious higher-dose toxicity, i.e. critical effects driving toxicity and

risk assessment. In addition to the challenges associated with dose-response modeling of high dimensional data such as global gene induction data, modeling or interpreting in vitro studies for nanomaterials have the additional challenge of accounting for particle size or density dependent differences in delivery (dose) to cells in culture. In this work, we combine regulatory network modeling, particokinetic modeling, and in vitro-in vivo extrapolation to develop a predictive dose-response model for nanoparticle induced immune response in RAW 264.7 macrophages. Gene expression profiles were evaluated in cells exposed in vitro for 24 hours to 10 nm, 300 nm and 500 nm carboxylated amorphous silica nanoparticles at three doses between 5 and 1000 μg/ml. Gene regulatory networks were inferred from gene expression profiles. Dose (particle surface area)-time vectors were constructed using a kinetic model of particle diffusion and sedimentation in vitro. Combining dose-time vectors across particle types, a quantitative model of the gene regulatory network was fit to the gene expression data. The resulting model was in good agreement with the dose and time dependencies in gene expression and could be used to predict no effect levels for gene expression. Extrapolated to equivalent human exposures, response and no effect levels were very high, suggesting that either lower environmental exposures to amorphous silica would be below a critical effect level, or that in vitro systems may not be appropriate for assessing the risks associated with nanoparticle exposure.

S 1718 TIME-DEPENDENT CHANGES IN THE TRANSCRIPTOME: AN APPROACH FOR IDENTIFYING TRANSCRIPTIONAL PROFILES ASSOCIATED WITH THE ONSET OF CHRONIC PHENOTYPIC CHANGES.

D. C. Wolf. *NHEERL/ORD, U. S. EPA, Research Triangle Park, NC.*

Although it is a basic tenet of toxicology that "the dose makes the poison", it is also true that it takes time for the biological processes that result in an adverse effect to accumulate. With the advent of high density data such as transcriptional profiling, time-dependent changes in biological processes at the cellular and molecular levels can be characterized and correlated with morphologic changes that develop with persistent exposure to a toxic chemical. Typical studies are designed such that histologic examination and transcriptional profiling are evaluated at a single time point. The evaluation of these endpoints over a broader span of time with repeated exposures provide insight as to the coordinated changes that occur relative to the advancing phenotype and the cellular biology that has adapted to support the altered structures. Examples using the urothelium lining the urinary bladder and nasal respiratory epithelium will be presented to illustrate time-dependent changes in transcriptional profiles of differentially expressed genes that can be further correlated with associated tissue responses. These reported changes also suggest that a chronic phenotype is associated with a chronic transcriptional profile. With appropriately designed studies, the time-dependent transitions from an acute transcriptional profile to a profile associated with chronic phenotypic changes can be determined.

S 1719 FORMALDEHYDE: DOSE-DEPENDENT TRANSITIONS FOR AN ENDOGENOUS COMPOUND WITH HIGH DOSE CARCINOGENICITY.

M. E. Andersen. *Computational Biology, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Formaldehyde (FA) is a normal byproduct of cellular metabolism, present in tissues at several hundred micromolar. At about 1 ppm, it is a nasal irritant. In rats, inhalation exposures of 6 ppm and higher for 6 hr/day, 5 days/week for 2 years caused nasal squamous cell carcinoma. Our research has developed a pharmacokinetic (PK) model for tissue formaldehyde acetal (FAcetal) and glutathione (GSH) in nasal epithelium and also measured whole genome microarray responses following daily 6 hr exposures to 0, 0.7, 2, 6, 10 and 15 ppm for 1, 4 or 13 weeks. A group or Sensitive Response Genes (SRGs) included thiol transport, thiol reduction, inflammatory signaling, and growth factor activation. These gene changes seen near the irritancy threshold are expected to be associated with decreasing free GSH as estimated by the PK modeling. At 6 ppm, genomic effects on cell cycle, p53, and stem cell signaling produce an environment conducive to malignant transformation. At these concentrations, FAcetal, the reactive form of FA in tissues, is rapidly increasing over its normal background concentrations. With FA, dose dependent transitions include threshold behaviors with an existing background FAcetal, a shift to irritancy with some adaptation, and finally overt toxicity and carcinogenicity with saturation of GSH by binding with FAcetal and increases in cellular acetal. Studies with endogenous compounds, such as FA, should be especially useful in establishing dose-dependent transitions and improving human health risk assessment defaults.

S 1720 ENVIRONMENTAL EPIGENOMICS AND RISK ASSESSMENT: WHAT CONSTITUTES AN ADVERSE EFFECT AND ISSUES OF NONLINEARITY.

D. C. Dolinoy. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

It is increasingly recognized that exposure to chemical, nutritional, and behavioral factors alters gene expression and affects health and disease by not only mutating promoter and coding regions of genes, but also by modifying the epigenome — modifications to DNA that confer an additional layer of heritable gene regulation that lead to disease when deregulated. Until recently, most attempts to elucidate environmental effects on the epigenome were either candidate gene driven or restricted in dose-response. Emerging advances in epigenomic and high-throughput quantitative epigenetic technologies now allow for the identification of the constellation of genomic loci with altered epigenetic status following dose-dependent exposures. Questions remain, however, in how best to define an adverse epigenetic effect and how to deal with non-linearity in epigenetic responses. First, like transcriptomics approaches, unbiased epigenomic mapping often reveals both increases and decreases in epigenetic marks, including DNA methylation, and disentangling the appropriate mechanisms underlying these effects is challenging. Second, the mouse is a tractable and popular model for human diseases; however it and other animal models may not be appropriate choices for modeling the potential impact on the human genome if the repertoire of epigenetically labile genes in mammals is markedly species dependent. Finally, even when an environmentally-induced epigenetic change is detected, it may be difficult to define the critical effect; for example unlike genetic mutations, epigenetic profiles are potentially reversible. Drawing upon data from multiple epigenomics platforms and focusing on bisphenol A (BPA) as a representative environmental exposure, we will explore dose-dependent methylation effects and associated perturbations in cellular interaction pathways. Epigenomic data from animal models, human clinical samples, and epidemiological studies now indicate that BPA-induced alterations vary between species and across dose, a revelation that should be considered in risk assessment.

S 1721 INTERPRETING DOSE-RESPONSE INFORMATION ON INTERMEDIATE STAGES OF CAUSAL CASCADES IN TOXICITY MODE OF ACTION.

L. R. Rhomberg. *Gradient, Cambridge, MA.*

Increasingly, instead of testing only apical endpoints, we are able to investigate dose-related patterns of changes in the underlying causative processes, including alterations at the cellular, physiologic, and gene-expression levels that may result from chemical exposure. New-technology testing allows multivariate response evaluation, greater sensitivity, and practical testing of more doses and conditions, but it requires new modes of interpretation regarding what changes are to be considered adverse, and questions are raised about how small changes in continuous underlying factors (and their observed dose-response patterns) translate into discrete changes from healthy to diseased states at the whole-organism level (and their dose-response patterns). I propose going beyond the key events in a mode-of-action framework as merely observable signposts to recognize a cascade of causative processes, with the outputs of earlier processes constituting the causes of later ones. Discrete changes of state as a consequence of continuous variation in causal factors can be seen as either control processes (from a systems-theory viewpoint) or failure modes of accommodation processes (from a catastrophy-theory viewpoint). Casting health and disease as emergent properties of complex interacting networks of causal processes provides a perspective on how to approach definition of adversity and interpretation of dose-response information at the gene-expression level.

W 1722 NONCLINICAL TO CLINICAL ABUSE LIABILITY ASSESSMENT OF DRUGS: CURRENT PRACTICES, CHALLENGES, AND IMPACT OF RECENT REGULATORY GUIDANCES.

B. Gemzik. *Discovery Toxicology, Bristol-Myers Squibb Company, Princeton, NJ.*

An increased level of attention has been focused on the assessment of abuse liability for drugs that affect the central nervous system as a consequence of recent regulatory guidances from the EMEA, ICH, as part of M3(R2), and FDA (draft). It is therefore critical to understand the current issues facing the pharmaceutical research and regulatory communities in the evaluation of new drug candidates for abuse and dependence potential. Nonclinical and clinical evaluations each contribute to the assessment of a drug for class scheduling, and to the information in the product label. Animal models for the study of drugs with pharmacologic mechanisms known to be associated with abuse are well characterized, and clinical trial protocols enrolling experienced users of abused drugs have been widely utilized in

risk assessment. However, unique challenges are encountered in both nonclinical and clinical discovery and development settings when evaluating drugs with novel mechanisms of action. In recognition of the need for a broad and integrated understanding in the toxicology community, this symposium will blend current topics in this field of growing interest, and present the scientific background and challenges in the context of the emerging regulatory landscape. Speakers will discuss the translational aspects of nonclinical and clinical findings to regulatory and scheduling processes. Emphasis will be placed on presentation and discussion of issues, with specific examples, that bridge these areas of interest and enhance overall awareness of the topic.

W 1723 NEUROPHARMACOLOGY OF DRUGS OF ABUSE AND IMPLICATIONS ACROSS DIVERSE DRUG DISCOVERY PROGRAMS.

D. R. Compton. *Preclinical Safety - Toxicology, sanofi-aventis U.S. Inc., Bridgewater, NJ.* Sponsor: B. Gemzik.

This presentation will provide a brief overview of the neuroscience related to the abuse of drugs in general and with special emphasis to issues specifically cited in the January 2010 US FDA Draft Guidance for Industry Assessment of Abuse Potential of Drugs, such as: a) the "direct and indirect actions" of drugs on "neurotransmitter systems associated with abuse potential" including dopamine, norepinephrine, serotonin, gamma-aminobutyric acid, acetylcholine, opioid, N-methyl-D-aspartate, and cannabinoid; b) receptor and/or neurotransmitter systems related to abuse potential not cited in the guidance; and c) the application of early nonclinical data to the development process for various categories of potential products (eg, CNS-targeted therapy, non-CNS-targeted therapy, biologics, oncologic agents); and d) the neurotoxic effects of acute and chronic drug use or abuse.

W 1724 ANIMAL MODELS FOR ASSESSMENT OF ABUSE POTENTIAL: TRANSLATION TO CLINICAL EVALUATION.

M. J. Kallman. *Covance Inc., Greenfield, IN.*

The emphasis of this presentation will focus on descriptions of the preclinical models and application of non-clinical data to clinical risk assessment of potential for abuse. Identification of strengths and weaknesses of the primary three models (drug discrimination, self-administration, and drug dependence/withdrawal)that are used for these non-clinical evaluations will be presented with consideration of some of the other models that may have utility for specific agents. The impact of species selection for the abuse liability package based on toxicology profile, pharmacokinetics, metabolite profile, and receptor conservation can also improve risk assessment.

W 1725 DESIGN AND INTERPRETATION OF CLINICAL TRIALS EVALUATING ABUSE POTENTIAL OF DRUGS: WHERE ARE THE GAPS?

M. Sokolowska. *Grünenthal USA, Bedminster, NJ.* Sponsor: B. Gemzik.

Abuse potential of drugs is evaluated based on data collected throughout the drug development program. The clinical assessments incorporate analyses of the human abuse potential study in addition to examination of adverse events of special interest and aberrant behaviors observed in Phases I – III. Even though the FDA Draft Guidance for Industry: Assessment of Abuse Potential of Drugs (Jan 2010) clarified some aspects of these assessments, further gaps have been identified. This presentation will focus on issues associated with the human abuse potential study design, specifically on the selection of subjects' population, types of controls, doses of investigational drug and controls, as well as the selection of outcome measures and parameters. Impact of various factors on data analysis including the contribution of the primary versus secondary outcome measures and differentiation between statistically significant versus clinically important differences will be examined. Additionally, methodology to evaluate adverse events and aberrant behaviors will be discussed.

W 1726 CURRENT CHALLENGES IN ABUSE LIABILITY ASSESSMENT: DRUGS WITH NOVEL MECHANISMS OF ACTION.

K. E. Cannon. *Global Safety Pharmacology CoE, Pfizer, Inc., Groton, CT.* Sponsor: T. Brabham.

Over the recent years, there has been a marked increase in the number of drug targets being pursued in the pharmaceutical industry with novel mechanisms of action that have either intended or unintended central nervous system activity. These novel targets pose unique scientific challenges for preclinical abuse liability assessments because any underlying basis for abuse potential may be entirely unknown. Thus, preclinical abuse assessments now present increasing challenges during the design, analysis, and interpretation of specific behavioral studies. In addition to a thorough evaluation of the target, several aspects of the compound profile including its secondary pharmacology (i.e. receptor binding profile and in vitro functional activity), in vivo pharmacodynamic effects, and pharmacokinetic profile must be carefully integrated and assessed. These properties will shape numerous facets of the study design, such as the model(s) to be assessed, species selection, parameters to be measured, and choice of positive comparator, in order to provide a comprehensive preclinical abuse liability profile of these novel mechanisms.

W 1727 REGULATORY AND SCHEDULING: PROCESSES AND CURRENT CONCERNS.

M. Klein. *CDER, U.S. FDA, Silver Spring, MD.* Sponsor: B. Gemzik.

The Food and Drug Administration issued a Draft Guidance for Industry on Assessment of Abuse Potential of Drugs in January 2010, in response to public health concerns related to availability and abuse of drugs. The guidance is intended to assist sponsors who are developing drug products with the potential for abuse on labeling issues and scheduling requirements under the Controlled Substances Act. Drugs with abuse potential generally include drugs that affect the central nervous system, drugs that are chemically or pharmacologically similar to other drugs with known abuse potential, and drugs that produce psychoactive effects such as sedation, euphoria, or mood change. The current FDA perspective on the guidance and related concerns will be discussed.

W 1728 RISK ASSESSMENT FOR PROTEINS INTRODUCED INTO GENETICALLY MODIFIED (GM) CROPS.

B. G. Hammond. *Product Safety Center, Monsanto Company, St. Louis, MO.*

In 2009, there was 330 million acres of genetically modified (GM) crops planted in 25 countries. Despite rapid adoption, planting of GM crops remains controversial in some countries. GM crops undergo comprehensive food safety assessment before commercialization. This includes proteins introduced into the crop to achieve desired technical effects. One of the safety questions asked is if the introduced proteins (IP) have a history of safe use (HOSU) in food. For registration of GM crops in Europe, if there is no reliable safety information, IP without a HOSU must be tested in a 28 day repeat dose toxicity study. Thus we will explore appropriate information that might resolve safety concerns for IP without a HOSU. Advances in the field of molecular biology over the last 30 years have increased our ability to modify the structure, stability, and activity of proteins of interest. These modified proteins may not have a HOSU. However, absence of HOSU does not mean absence of safety. Protein engineering and evolution studies suggest that changes in amino acid sequences of proteins not related to those with known toxicological hazards (i.e., toxins or allergens) will not make a protein potentially hazardous de novo. Modifications often exert little effect on biological function, and some substitutions are deleterious to protein structure and function. In regards to dietary risk assessment of proteins introduced into GM crops, risk assessors have generally made the highly conservative assumption that the IP remains functionally intact during the processing of the crop into human food fractions. However for crops such as corn or soy which are extensively processed, the IP that have been tested do not survive processing functionally intact. This information could be relevant to resolving concerns about the safety of IP that do not have a HOSU. However, if the IP is related to known mammalian toxins, remains functionally intact after processing or is not susceptible to digestion, then further toxicology testing may be needed.

W 1729 NATURE'S BALANCING ACT: EVOLUTIONARY CHANGES IN PROTEIN FAMILIES.

J. M. Jez. *Donald Danforth Plant Science Center, St. Louis, MO.* Sponsor: B. Hammond.

The dynamic interplay of an organism's DNA, metabolism, protein structure, biochemical function, and environment drives the evolution of new proteins. The requirement of an organism to maintain fitness balances the changes that drive molecular evolution of protein structure and function. The dominant genetic mechanisms that lead to changes in protein function are: i) gene duplications; ii) divergence of gene sequences to produce proteins with modified sequences, structures, and functions; iii) selection of new sequences with value to organism fitness; and, in many cases, iv) combination with other genes to continue the process of evolution. Although DNA plasticity is central to evolution, the evolution to "new"

three-dimensional structures and biochemical function is gradual, if it occurs at all, as any sequence changes are balanced by the need to maintain organism survival and the intrinsic physical properties of the protein for its function. Protein engineering and evolution studies suggest that changes in sequences of proteins not related to known toxicological hazards (i.e., toxins or allergens) will not make a protein potentially hazardous de novo and will likely exert little effect on biological function, even though some substitutions are deleterious to protein structure and function.

W 1730 ENGINEERING PROTEINS TO IMPROVE BIOLOGICAL FUNCTION.

S. J. Franklin. *Protein Technologies, Monsanto Company, Cambridge, MA.* Sponsor: B. Hammond.

Advances in the field of molecular biology over the last 30 years have greatly enabled our ability to modify the structure, stability and activity of proteins of interest. The growth of this "protein design" discipline has resulted in the modification of numerous proteins resulting in beneficial therapeutic agents, biochemical reagents, and even enhanced agricultural crops. The activities of such engineered proteins are determined by the details of their three-dimensional structures. The tools of molecular biology allow us to refine structure as appropriate, under the control of the laws of physics. When coupled to high-throughput techniques and computational analyses, protein design can create hundreds of thousands of proteins, some of which have improved properties. At the same time, screening and evaluation of products is an integral part of the process, to minimizing creating proteins with no function or with less desirable activities. The convergence of these abilities provides great possibilities to create products that positively impact human health and nutrition in numerous ways. This talk will highlight the underlying biophysical principles that guide protein behavior, and advances in protein design that allow the generation of custom proteins with improved, well-characterized functional properties.

W 1731 SAFETY ASSESSMENT OF NOVEL PROTEINS.

J. L. Kough. *Biopesticides and Pollution Protection Division, Office of Pesticide Programs, U.S. EPA, Washington, DC.* Sponsor: B. Hammond.

The Environmental Protection Agency registers all pesticides sold in the U.S. under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and establishes safe residue levels under the Federal Food Drug and Cosmetic Act (FFDCA). The advent of plant transformation technology has led to the production of transgenic plants with new pesticidal properties. These biopesticides are termed Plant-Incorporated Protectants (PIPs). This category includes plants produced by introduction of traits that would not normally be available by traditional plant breeding methods with sexually compatible relatives. An example would be the integration of bacterial gene sequences into corn for insect resistance. Most PIPs registered to date have been proteins. Prior to registration, each PIP is examined to decide what data are needed to insure that a reasonable certainty of no harm to man and the environment will result from its use. In general, details of the gene construct and expression levels of the introduced pesticidal substance, as well as information on the amino acid similarity to toxins and allergens, the digestibility and stability of proteins produced and toxicity profiles for humans and non-target species are requested from the registrant.

W 1732 EFSA'S UPDATED STRATEGY FOR THE RISK ASSESSMENT OF GM PLANTS AND DERIVED FOOD/FEED.

H. A. Kuiper. *Chair EFSA GMO Panel, European Food Safety Agency (EFSA), Parma, Italy.* Sponsor: B. Hammond.

The EFSA Guidance Document for the risk assessment of genetically modified plants and derived food and feed provides guidance for the preparation and presentation of applications submitted within the framework of Regulation (EC) 1829/2003 on GM food and feed, and of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMOs). The EFSA's GMO Panel regularly reviews its guidance to take account of scientific developments and of experience gained through the risk assessment process. In 2008 the Panel updated its guidance document in the light of experience in the risk assessment of GMO applications and of the outcome of the Panels' self-tasking activities. The changes with respect to the risk assessment of food/feed derived from GM plants will be highlighted in this presentation. Among others the present draft includes updated guidance on: (i) the experimental design of field trials and the statistical analysis of the collected data, (ii) toxicity testing of newly expressed proteins and new constituents other than proteins, and (iii) role of animal feeding trials with whole GM plant material - based on the report of a related EFSA self tasking activity. The updated guidance document was also used by the Commission and the Member States as a basis for the preparation of EC Guidelines.

W 1733 RISK ASSESSMENT RECOMMENDATIONS FOR INTRODUCED PROTEINS.

B. G. Hammond. *Product Safety Center, Monsanto Company, Saint Louis, MO.*

ILSI/IFBiC Task Force 10 was formed to address the role of mammalian toxicology studies in assessing the safety of the introduced proteins (IP) and the whole foods from GM crops. This task force is composed of academic, industry and regulatory agency experts from various world areas. This group addressed in part the history of safe use (HOSU) concept in food which is a component of the dietary risk assessment of IP. If a protein has a HOSU in food, this can support the weight of evidence of its safety for consumption. Some IP will not have a HOSU however, but that does not necessarily mean that their safety for consumption is uncertain. Some IP are structurally/ functionally related to proteins that have a HOSU. Evolutionary divergence of protein families (e.g., enzymes) across different species has shown that there is considerable variation in amino acid sequence/content, yet the catalytic site is highly conserved and the enzymes have related functions. Based on our understanding of protein evolution and learnings from protein engineering, there is no evidence that changes in amino acid content/sequence impart toxic effects to proteins that are not known to be toxic. A second aspect of the dietary risk assessment deals with potential dietary exposure. Crops such as corn and soybeans are extensively processed into a variety of human foods that often leads to denaturation and loss of protein function. This has also been demonstrated for IP, such as enzymes and insect control proteins. IP proteins that have been tested are generally heat labile and are likely to be denatured by cooking during normal processing of corn or soy into human food. Thus, toxicology testing of IP without a HOSU may not be needed if the proteins are structurally/functionally related to those that have HOSU, and/or there would be negligible dietary exposure to functionally active IP as they are expected to be denatured during processing of corn or soy into human foods.

W 1734 THE SPECTRUM OF SYSTEMS BIOLOGY.

L. Burgoon[1] and W. Mattes[2]. [1]*U.S. EPA, Durham, NC and* [2]*PharmPoint Consulting, Poolesville, MD.*

What is Systems Biology? While being hailed as the most promising approach for creating breakthroughs in scientific understanding in the wake of the 'omics crush, systems biology is a phrase used to describe a multitude of approaches to understanding and/or predicting biological responses to stimuli. Decades ago systems biology invoked quantitative metabolic flux modeling, while today it is used to describe pathway analysis of 'omic data, quantitative signaling network modeling, the integration of multiple 'omic data, and many other approaches. Our panel of experts are poised to discuss their divergent views of systems biology and will describe how their research addresses a holistic solution to biologic data. Before concluding a panel discussion will convene that will provide a more global (i.e., systems) view of systems biology.

W 1735 BIOSIMULATION OF DRUG-INDUCED LIVER INJURY.

H. J. Clewell[1], B. A. Howell[1], Y. Yang[1], S. Q. Siler[2], R. Ho[2], R. Kumar[2], A. H. Harrill[1], M. E. Andersen[1] and P. B. Watkins[1]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [2]*Entelos, Inc., Foster City, CA.*

A biosimulation model is being developed to provide a quantitative description of the key processes involved in drug-induced liver injury: metabolism, reactive metabolite formation, glutathione depletion and resynthesis, ROS formation, disruption of energy homeostasis, mitochondrial dysfunction, necrosis, apoptosis, and tissue regeneration. The initial development of the platform has focused on acetaminophen (APAP) and includes a whole-body physiologically based pharmacokinetic (PBPK) model for APAP and its metabolites in the mouse, rat, and human. The PBPK model is linked to a pharmacodynamic (PD) model describing the reaction of APAP-derived N-acetyl-p-benzoquinone imine (NAPQI) with glutathione and the resulting depletion and resynthesis of glutathione. The formation of NAPQI-adducts with cellular proteins and increased oxidative stress resulting from glutathione depletion are linked to cellular damage and response. This PBPK/PD

model of APAP metabolism and glutathione depletion has been used to investigate the extent to which differences in metabolism and glutathione homeostasis explain interspecies differences in susceptibility to APAP toxicity. As compounds with other modalities of DILI are added to the platform, the resulting model will serve as a flexible biosimulation platform to enhance drug development by quickly identifying drug candidates that are likely to be associated with idiosyncratic hepatic toxicity, and to guide personalized medicine by determining patient characteristics associated with a greater likelihood of adverse liver responses.

W 1736 DEFINING SYSTEMS BIOLOGY IN A MODE-OF-ACTION CONTEXT.

S. Edwards. *U.S. EPA, Research Triangle Park, NC.*

There are many competing definitions for the term systems biology with much of the ambiguity originating from the revolution in molecular biology techniques at the turn of the century. The sequencing of the human genome and advent of techniques for monitoring transcripts, proteins, and metabolites on a global scale opened the door for a new world of molecular systems biology. In this new era, most definitions of systems biology contain most (if not all) of the following elements: global measurements of biological molecules to the extent technically feasible, dynamic measurements of key biological molecules to establish quantitative relationships among them, and experimental designs which perturb the system in specific ways to determine these relationships. This presentation will discuss how this these components can be used to develop a model for disease based on an interconnected network of molecular, cellular, and organism-level events. These disease networks can then serve as the framework for a network-based description of mode of action. Approaching the problem from this perspective provides a biologically-based mechanism for incorporating other factors affecting risk such as genetic susceptibility, life stage, and pre-existing diseases. It also enhances the ability of more traditional biological modeling approaches to lay the groundwork for toxicity pathway-based risk assessment. The approach will be illustrated using examples from both human health and ecological research. [This abstract does not necessarily reflect the view of the Environmental Protection Agency.]

W 1737 PHARMGKB: THE PHARMCOGENOMICS KNOWLEDGE BASE.

T. E. Klein. *Department of Genetics, Stanford University Medical Center, Stanford, CA.* Sponsor: L. Burgoon.

The mission of the PharmGKB is to collect, encode, and disseminate knowledge about how human genetic variation affects drug response. Established in 2000, PharmGKB has become the pre-eminent resource for pharmacogenomics information. It was one of the first "post-genome" resources with information about both genotype and phenotype, and has now become a focal point for data sharing. Via literature review, the PharmGKB team curates primary data, and annotates gene variants and gene-drug-disease relationships, The integration of genotype, phenotype, and drug pathways allows for a systems level analysis and hypothesis generation on new gene-drug interactions and effects.

W 1738 THE INTERSECTOME: INTEGRATION OF KNOWLEDGE IN SYSTEMS BIOLOGY FOR HYPOTHESIS GENERATION.

A. Ma'ayan. *Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY.* Sponsor: L. Burgoon.

I will discuss how we utilize data collected from the public domain, describing regulatory interactions in mammalian cells, to analyze results from experiments that profile cells using a variety of cutting edge genome-wide profiling technologies. The results from our analyses produce rational hypotheses for further experimental validation as well as provide a global view of cell regulation across multiple layers. Specifically, I will show GATE, a program we developed for the analysis of time-series expression data used to analyze stem cell differentiation. I will also demonstrate Genes2Networks a program we developed and used to predict components and pathways essential for CB1R induced neurite outgrowth of Neuro2A cells. Genes2Networks was also used to predict a novel disease gene that causes Noonan syndrome. Finally, I will discuss our tools KEA for the analysis of SILAC phosphoproteomics, and ChEA for the analysis of gene expression data using a ChIP-X database. I will conclude with a proposition of ideas about developing robust theoretical models for candidate drug/gene/protein rankings for functional experimental validation.

W 1739 INTEGRATED ANALYSIS OF DISTINCT MOLECULAR AND PHENOTYPIC DATA TYPES IN XENOBIOTIC RESPONSE MODELING.

R. J. Brennan. *Toxicology, GeneGo Inc., San Jose, CA.*

Signaling and metabolic pathways acting within and between cells in an organism may be considered as integrated circuits working to maintain cell growth or homeostasis based on inputs from external influences and other, connected pathways. The components of these circuits comprise DNA, RNA, proteins, enzymatic activities, small molecules, ions etc., and in order to fully understand the circuit, or to "reconstruct the system" it is necessary to consider all of it's components as well as the connections (interactions) between them. These concepts are the basis of the systems biology approach to biological pathway analysis and network reconstruction. In recent years this approach has been successfully used to integrate data of different types, and from different sources, on multiple types of circuit component – genes, mRNA, proteins, metabolites, microRNA, to obtain a more precise and comprehensive understanding of how disruptions to the circuitry can lead to disease or toxicity. Gross damage to the circuitry by removal or functional impairment of certain components may impact the functioning of the circuit. Individual differences in the system, arising through sequence variations in the DNA template for individual components, also may affect the response of the circuit to normal stimuli or to xenobiotic interference. These variations manifest as different susceptibilities to disease, responses to therapeutic intervention or risks of chemical toxicity. The integration of data on the functional consequences of sequence variation to the systems biology circuit model will be critical to complete understanding of system malfunction or failure in disease or toxicity, and to the successful implementation of personalized medicine.

PL 1740 ZEBRAFISH: A PREDICTIVE MODEL FOR ASSESSING CANCER DRUG-INDUCED ORGAN TOXICITY.

L. D'Amico[1], C. Li[1], E. Glaze[2], M. Davis[2] and W. L. Seng[2]. [1]*Phylonix, Cambridge, MA and* [2]*NCI, NIH, Bethesda, MD.* Sponsor: P. McGrath.

Toxic effects of 4 cancer compounds on CNS, heart, liver, and kidney were visually assessed in live, transparent zebrafish and compared to effects in mammals and humans. Compounds, tested blinded, included: 1) 17-DMAG, an HSP90 inhibitor in Phase I clinical trials for lymphoma, 2) paclitaxel, a microtubule stabilizer approved for breast cancer, 3) SMA-838, a transcriptional inhibitor, and 4) zebularine, a DNA methyltransferase inhibitor which has exhibited antitumor effects in preclinical studies. After adding compounds directly to fish water, LC10 and LC50 were estimated and 6 concentrations below LC10 were then used to treat 2 or 4 day post fertilization zebrafish. Results showed that 17-DMAG induced defects in zebrafish CNS, heart, liver, and kidney and cell death in CNS, heart, and liver. In comparison, in rat and dog studies, only liver specific toxicity was observed. Similar to results in zebrafish, human clinical trial data showed that 17-DMAG induced CNS, heart, liver and kidney toxicity. Due to low solubility, at concentrations up to 2 μM, paclitaxel, induced CNS toxicity, similar to data reported in human clinical trials. In contrast, in rats, no toxicity was identified in test organs. SMA838 caused both morphological defects and cell death in zebrafish CNS and liver. In comparison, in rats, no toxicity was observed in test organs. However, dog studies identified liver and kidney toxicity. No toxicity was observed in zebularine treated zebrafish, similar to results in preclinical studies. This small targeted study highlights the utility of using transparent zebrafish for assessing drug induced organ toxicity eliminating the need for surgical procedures. Advantages of using zebrafish as an animal model for assessing drug induced toxicity include: small amount of drug required, easy drug treatment directly in the fish water, short treatment time, visual assessment in transparent animals without the need for surgery and laborious processing, statistically significant number of animals per test, and low cost.

PL 1741 MANIPULATION OF THE WNT/β-CATENIN PATHWAY BY PROTOTYPIC INHIBITORS COMPARED TO MORPHOLINO KNOCKDOWN IN ZEBRAFISH.

S. M. Eddy, D. B. Stedman and M. D. Aleo. *Drug Safety, Pfizer Global Research and Development, Groton, CT.*

Manipulation of the Wnt/β-Catenin signaling pathway can be demonstrated in Zebrafish (ZF) by treatment with prototypic inhibitors or Morpholino knock down of TCF4 or β-Catenin. Expression analysis following treatment with prototypic compounds FH535, IWR-1, XAV939, and SB415286 confirmed their effects on the Wnt pathway. Phenotype following inhibitor treatment was compared to that after TCF4 or β-Catenin Morpholino injection. Wild type Zf embryos were treated with FH535, IWR-1, XAV939, and SB415286 at 6, 24, 48, and 72 hours

post fertilization (hpf) for 5 days when they were scored for acute lethality and developmental defects. Six hpf embryos were treated with compound and RNA isolated for RT-PCR at 10 or 24 hpf to confirm downstream canonical Wnt target inhibition (Axin2 and Cyclin D1) using RPL13a as an endogenous control. Non-canonical Wnt Planar Cell pathway VANGL2 expression was also measured. FH535, a transcriptional Wnt pathway inhibitor, suppressed Axin2 and Cyclin D1 expression at 10 and 24 hpf. IWR-1, a Wnt/tankyrase inhibitor, induced malformations while showing no change in Axin2 or Cyclin D1 expression. XAV939, a more selective tankyrase inhibitor, induced malformations such as micropthalmia, misshapen head and heart, and tail defects but had little expression change. SB415286, a GSK3β inhibitor, increased in Axin2 and Cyclin D1 expression at 10 hpf. Wnt/PCP pathway was also evaluated by measurement of VANGL2 expression. Following IWR-1 treatment VANGL2 expression increased by 2 fold at 24 hpf and by 4 fold at 5 days. TFC4 and β-Catenin Morpholinos were injected into embryos at the 2 cell stage and evaluated for defects/mortality at 48 hpf. Both the TCF4 and β-Catenin Morpholinos had severe phenotypic effects. In summary, prototypic inhibitors of the Wnt/β-Catenin pathway have measurable effects on Wnt target gene expression and phenoptypic malformations are similar though less extreme than that of Morpholino knock down in the developing Zf embryo.

PL 1742 ASSESSMENT OF DRUG-INDUCED, SEIZURE-LIKE MOVEMENT IN ZEBRAFISH.

D. Park, J. Meidenbauer, W. L. Seng and P. McGrath. *Phylonix, Cambridge, MA.*

Zebrafish has been shown to be a predictive animal model for assessing drug induced seizure-like activity. In a recent study, using a video camera, we recorded swim movement of 6 dpf zebrafish for 60 min after treatment with 9 compounds known to induce seizures in mammals including: aminophylline hydrate, 4-aminopyridine, amoxapine, bicuculline methoiodide, enoxacin, methoxychlorate, pentylenetetrazole, picrotoxin, and strychnine hemisulphate, and a negative control, lidocaine. Using video recordings, we quantitated high speed (>20 mm/sec) movement which correlated with seizure-like activity. Distance moved (cm) at high speed in 1 min intervals was plotted against recording time. As shown by each time course curve, 7 test drugs, aminophylline hydrate, 4-aminopyridine, amoxapine, methoxychlorate, pentylenetetrazole, picrotoxin, and strychnine hemisulphate, induced peak movement. In addition, although the kinetics of induction and the duration of seizure-like activity varied by drug, a dose-dependent increase in high speed movement was observed for 7 test drugs. Cumulative distance moved (cm) at high speed after drug treatment was also plotted against time and 8 test drugs, aminophylline hydrate, 4-aminopyridine, amoxapine, bicuculline methoiodide, methoxychlorate, pentylenetetrazole, picrotoxin, and strychnine hemisulphate, showed a dose-dependent change. Drugs known to cause seizure effects in mammals showed similar effects in zebrafish. Since the assay correctly predicted results for 8 of 9 positive drugs, sensitivity was 89% (excellent). Our data support use of zebrafish model for assessing drug induced seizures. This functional motility assay can be combined with other zebrafish assays to assess drug effects on CNS. Advantages of zebrafish for safety and toxicity studies include: large number of embryos per mating, drug delivery directly in the fish water, small amount of compound required (μM vs mM) and statistically significant number of animals per experiment.

PL 1743 VALIDATION OF AN AUTOMATED SCREENING ASSAY BASED IN A ZEBRAFISH MODEL FOR THE DETECTION OF COMPOUNDS THAT INHIBIT ANGIOGENESIS.

C. Quevedo, C. Callol and A. Letamendia. *Scientific, Biobide, San Sebastian, Guipuzkoa, Spain.* Sponsor: S. Tripathi.

Angiogenesis plays an essential role in tumor growth and metastasis, so it is a well-established therapeutic target. The zebrafish is the only vertebrate model compatible with the paradigms of drug discovery. The small size and transparency of zebrafish embryos make them amenable for the automation necessary in high-throughput screens. We have developed an automated, quantitative screening assay for the discovery of antiangiogenic compounds using transgenic zebrafish embryos that express Cop-GFP under the control of the endothelial specific flk-1 promoter. The assay includes automated methods for embryo dispensation, compound delivery and embryo imaging. The angiogenic vessels that perfuse the trunk of the embryo (intersegmental vessels) at ~24 hours post-fertilization constitute the readout of the assay. To validate this method, a battery of known inhibitors for different targets implicated in angiogenesis (mainly Tyrosine kinase inhibitors) has being used. We can discriminate different and specific phenotypes related with vascular disruption (endothelial proliferation and migration and neovascular stabilization). Our data shows the suitability of a vertebrate animal model for drug discovery assays with libraries of multiple compounds as well as the capacity to visualize anti-angiogenic compounds with high specificity and sensibility in an in vivo model.

PL 1744 THE EFFECTS OF CHEMICALS IN THE U.S. EPA'S TOXCAST LIBRARY ON *CAENORHABDITIS ELEGANS* AND ZEBRAFISH DEVELOPMENT.

W. A. Boyd[1], M. V. Smith[2] and J. H. Freedman[1]. [1]*NIEHS, Durham, NC and* [2]*SRA International, Durham, NC.*

The need to characterize the potential toxicity of thousands of chemicals has led government organizations to explore the use of high-throughput in vitro screens and alternative model systems. These approaches are part of an effort to develop a tiered toxicity testing scheme. As part of this effort, the EPA developed a standardized collection of chemicals: the ToxCast Phase I library. This library is composed of 309 unique compounds, including pesticide active ingredients that have well-characterized mammalian toxicities. The nematode Caenorhabditis elegans and zebrafish Danio rerio are two popular model organisms used to study developmental biology that are also used as alternative toxicological models. The effects of the chemicals in the ToxCast library on zebrafish embryonic development and C. elegans larval growth were compared. The C. elegans growth assay monitors changes in nematode size following exposure to chemicals from the first through the last larval stage, while the zebrafish assay monitors malformations in larvae following exposure from 6h to 6d post-fertilization. The ToxCast library was screened for effects on C. elegans growth at seven concentrations (0.5 - 200 μM) and zebrafish development at eleven concentrations (0.001 – 80 μM). A high percentage of compounds caused developmental defects in zebrafish and decreased growth in C. elegans. A high concordance was observed between the nematode and zebrafish results. Over 40% of the compounds were toxic to both organisms and 30% were not toxic to either at the maximum concentrations tested. The class of pesticides with the highest number of toxic compounds included conazoles, pyrethroids, and organophosphates. The compounds' activities in both organisms were highly correlated with log octanol-water partition coefficients and molecular weights. These results can be compared with those obtained from rodent assays and high-throughput screens to assess their utility in tiered toxicity testing.

PL 1745 A PROTEOMIC ANALYSIS OF ARSENIC EXPOSED ZEBRAFISH (*DANIO RERIO*) SUGGESTS ALTERED LIPID METABOLISM.

P. Carlson[1] and R. J. Van Beneden[1, 2]. [1]*Graduate School of Biomedical Sciences, University of Maine, Orono, ME and* [2]*School of Marine Sciences, University of Maine, Orono, ME.* Sponsor: G. Mayer.

The zebrafish (*Danio rerio*), a well-established model of liver development and disease, was used to investigate differential protein expression following arsenic exposure. Several disorders have been linked to arsenic exposure including cancer, diabetes and lipid metabolism dysregulation, although the mechanisms of arsenic toxicity are still poorly understood. In vitro, arsenic has been shown to induce free radical formation as well as to interfere with the phosphorylation of a number of key regulatory proteins, including p53 and the epidermal growth factor receptor. Other studies have described altered gene expression and mitogenic signaling, but the effects on protein expression following arsenic exposure over a prolonged period have yet to be described. A bottom-up proteomics approach was employed to investigate arsenic induced alteration in the zebrafish liver proteome. Over 740 proteins were identified, with fewer than 2% showing differential expression. The data suggest that arsenic exposure altered the expression of proteins involved in lipid metabolism. Two key pathways have been identified, involving peroxisome proliferator-activated receptor gamma (PPARγ) and hepatocyte nuclear factor 4 alpha (HNF4α). PPARγ acts as a key regulator of adipocyte differentiation and blood glucose homeostasis; HNF4α is a transcription factor for several lipid metabolism enzymes and insulin. Altered expression of proteins in the PPARγ pathway include fatty acid binding protein (FABP), collagen 1A1 (COL1A1); enzymes altered in the HNF4α pathway include hydroxysteroid dehydrogenase like 2 (HSDL2) and N-acetylgalactosaminidase α (NAGA). These results are being confirmed by ongoing studies of the regulation of PPARγ and HNF4α.

PL 1746 LOCALIZATION OF MEGALIN ALONG ZEBRAFISH LATERAL LINE.

C. Doshna[1], C. Nykyforchyn[2], P. Burch[1] and M. D. Aleo[1]. [1]*DSRD, Pfizer, Groton, CT and* [2]*University of CT, Storrs, CT.*

Megalin is most abundantly located in renal proximal tubule & inner ear epithelial cells in mammals, acting as an endocytic receptor for aminoglycosides (AG) & other polybasic drugs that cause nephrotoxic & ototoxic effects in mammals. The presence of megalin has been shown in zebrafish (Zfish) embryos however, its potential role in the ototoxic response to AG & polymyxins has only recently been suggested through work by this lab. The fact that Zfish hair cells are both physio-

logically & morphologically similar to that of human hair cells, along with our studies, indicate that megalin is likely located in/near hair cells of the Zfish lateral line. Zfish exposure to AG & cisplatin resulted in a loss of fluorescently-labeled hair cells, corresponding to cell death. Furthermore, concomitant application of known megalin binding agents led to partial protection of drug-induced Zfish hair cell death. The purpose of this study is to determine whether megalin is located in/near Zfish hair cells as well as further investigate effectiveness of protective agents in preventing hair cell death & loss of fluorescence. Zfish at 5 days post fertilization (dpf) labeled with anti-megalin antibody showed patterns closely resembling neuromast structures seen with fluorescent labeling. These results show megalin is located in hair cell neuromast structures along the lateral line of Zfish. Zfish at 4 dpf were treated with polymyxin B, polymyxin B nonapeptide or gentamicin +/- poly-L-asparagine, labeled with the fluorescent dye YoPro1 & imaged using fluorescence microscopy to look for a loss of hair cell fluorescence vs. controls. Only partial protection was seen with pretreatment of megalin binding agent. Nephrotoxic cephalosporins that do not go through megalin were also tested & no loss of fluorescence was seen up to 100 μM, further supporting the conclusion that megalin plays a key role in the mechanism through which nephrotoxic drugs illicit an ototoxic effect in Zfish. Drugs that bind to megalin could conceivably be screened in Zfish using hair cell toxicity as a potential predictor for nephro- and ototoxicity in mammals.

PL 1747 PERSISTENT LIFE HISTORY EFFECTS OF ATRAZINE EXPOSURE IN ZEBRAFISH: A MULTI-GENERATIONAL STUDY ENCOMPASSING GENE EXPRESSION, FITNESS, AND EPIGENETIC EFFECTS.

S. Lewis[1], G. J. Weber[2], S. M. Peterson[2], J. L. Freeman[2] and M. S. Sepulveda[1]. [1]*Forestry and Natural Resources, Purdue University, West Lafayette, IN and* [2]*School of Health Sciences, Purdue University, West Lafayette, IN.*

Atrazine is one of the most widely used herbicides in the United States because it is highly effective against a wide spectrum of broadleaf and grass weeds and because it is relatively inexpensive. At present, the maximum contaminant level goals (MCLG) for atrazine, set by the U.S. EPA, is 3 ppb (parts per billion) for drinking water. The safety of atrazine exposure is currently being re-evaluated by the EPA due to recent studies linking exposure with cancer and endocrine disruption. Therefore, we are studying the lasting effects of a one-time developmental atrazine exposure in later life stages of the exposed individuals and in their offspring by evaluating differential gene expression, fitness, survival of offspring and hatching success. In a previous study using microarray technology, an exposure of embryonic zebrafish through 72 hours post fertilization (hpf) to 0.3 ppb, 3 ppb, or 30 ppb atrazine resulted in differentially expressed gene lists enriched with genes associated with neuroendocrine development, cell proliferation, and cancer development. From these gene set lists, a subset of candidate genes were chosen as genetic biomarkers for neuroendocrine functions and gene expression alterations confirmed with quantitative real-time PCR (qPCR) in the 72 hpf larvae. Lasting effects on gene expression of these same genes were also measured by qPCR in 33-day old juveniles and in sexually mature adults. Additionally, the effect of atrazine on sex ratio in the exposed generation (F0) and on the number of offspring and hatching success of the F1 was also measured. No alteration in sex ratio was observed, but the lowest atrazine treatment of 0.3 ppb resulted in decreased survivorship in the F1 generation. No effects on survivorship or hatching rate were observed in the offspring of the fish exposed to 3 ppb and 30 ppb atrazine.

PL 1748 MOLECULAR EVALUATION OF RABBIT CORNEAL OCULAR SULFUR MUSTARD INJURY: TOWARDS MECHANISMS OF PERSISTENT INJURY.

D. Milhorn[2], P. McNutt[1], T. Hamilton[1] and R. Lawrence[1]. [1]*USAMRICD, Aberdeen Proving Ground- Edgewood Area, MD and* [2]*Medical Research and Materiel Command, Fort Detrick, MD.* Sponsor: G. Rockwood.

Sulfur mustard (SM, bis-(2-chloroethyl) sulfide) is a highly reactive, bifunctional alkylating agent that covalently modifies DNA, proteins, and other macromolecules. Ocular SM exposure results in a severe acute injury involving inflammation and corneal erosions that rapidly resolve. Many victims subsequently undergo a delayed injury that severely compromises eyesight, characterized by corneal erosions, persistent edema and neovascularization. The etiology of these recurrent keratopathies is unknown. Diverse therapeutic approaches have failed to mitigate the delayed injury, suggesting that researchers need an improved understanding of the mechanisms underlying injury progression. We have developed a novel rabbit corneal vapor exposure model that exhibits acute and long-term sequelae commensurate with human clinical reports and characterized consistent and reproducible metrics of injury progression. Exposure to SM results in sloughing of corneal epithelium and stromal cell necrosis within 24 hr. The corneal lesion is re-epithelialized within 96 hr, and there is minimal evidence of injury by 1-2 wk. However, 75% of animals undergo a recurrent injury between 3-5 wk that is progressive and more severe than the acute injury, characterized by neovascularization, persistent immune cell infiltration, significant corneal edema and recurrent corneal erosions. For the first time we present correlations between ultrastructural and clinical aspects of the acute and recurrent injuries from 0-5 wk, correlating architectural changes in the corneal epithelium, basement membrane and stroma with the onset of recurrent sequelae. We evaluate these results in terms of injury mechanism(s) and novel, evidence-based therapeutic approaches as well as propose future research directions to identify the contribution of specific corneal tissues to SM injury.

PL 1749 INDUCTION OF AUTOPHAGY IN A MOUSE KERATINOCYTE CONSTRUCT MODEL BY THE VESICANT 2-CHLOROETHYL ETHYL SULFIDE; REGULATION BY MAP KINASES AND CAVEOLAE.

A. T. Black[1], R. P. Casillas[2], D. E. Heck[3], D. R. Gerecke[1], D. L. Laskin[1] and J. D. Laskin[4]. [1]*Rutgers University, Piscataway, NJ,* [2]*Battelle Biomedical Research Center, Columbus, OH,* [3]*New York Medical College, Valhalla, NY and* [4]*UMDNJ-RWJ Medical School, Piscataway, NJ.*

Sulfur mustard is a potent vesicant that causes inflammation, edema and blistering in the skin. These effects are associated with basal keratinocyte damage and cytotoxicity. Autophagy is a membrane-trafficking mechanism for protein and cytoplasmic organelle degradation and is enhanced in stressed cells. In the present studies, we analyzed the effects of the half mustard 2-chloroethyl ethyl sulfide (CEES) on the autophagic process in keratinocytes using a skin construct model in which cells are grown at an air-surface interface. CEES (100-1000 μM) caused a concentration-dependent increase in autophagy in keratinocytes as detected by flow cytometry in conjunction with acridine orange staining. CEES treatment also upregulated gene and protein expression of LC3B, beclin-1, and p62, proteins known to participate in autophagy, as well as caveolin-1, the primary structural protein of caveolae. Isolation of cytosolic and caveolar fractions from keratinocytes showed that LC3B and p62 were specifically localized within caveolae, while beclin-1 was found primarily in the cytosol of control cells, and in both cytosolic and caveolar fractions of CEES-treated cells. Disruption of caveolae using filipin (20 μM) or methyl-β-cyclodextrin (5 mM) markedly suppressed expression of LC3B, beclin-1 and p62 in CEES-treated keratinocytes. CEES also activated the JNK and p38 MAP kinase signaling pathways in keratinocytes. Treatment of the cells with inhibitors of JNK (SP600125, 20 μM) or p38 (SB203580, 10 μM) markedly suppressed expression of LC3B, beclin-1 and p62, as well as caveolin-1. Taken together, these data indicate that caveolae play a key role in CEES-induced autophagy in keratinocytes and that this process is regulated JNK and p38 MAP kinases. Supported by CA093798, ES004738, ES005022, GM034310 and AR055073.

PL 1750 ROLE OF CAVEOLAE IN REGULATING EXPRESSION OF HSP27 AND HSP70 FOLLOWING EXPOSURE OF HUMAN AND MOUSE SKIN MODELS TO THE VESICANT 2-CHLOROETHYL ETHYL SULFIDE.

J. D. Laskin[1], A. T. Black[2], P. J. Hayden[3], R. P. Casillas[4], D. E. Heck[5], D. R. Gerecke[2] and D. L. Laskin[2]. [1]*UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ,* [2]*Rutgers University, Piscataway, NJ,* [3]*MatTek Corporation, Ashland, MA,* [4]*Batelle Memorial Institute, Columbus, OH and* [5]*New York Medical College, Valhalla, NY.*

Dermal exposure to the vesicant sulfur mustard causes marked inflammation and tissue damage. Although sulfur mustard is a non-specific alkylating agent, basal keratinocytes appear to be a major target. In the present studies, mechanisms mediating sulfur mustard-induced skin toxicity were examined using a full-thickness human skin equivalent (EpiDerm-FT™) and a mouse keratinocyte skin construct model. In both models, treatment of keratinocytes at the air surface with 2-chloroethyl ethyl sulfide (CEES, 100-1000 μM) induced gene and protein expression of caveolin-1, a major component of caveolae which are membrane structural elements important in regulating intracellular signaling. CEES treatment also increased gene and protein expression for heat shock proteins 27 and 70 (Hsp27 and Hsp70). In the full-thickness human skin equivalent, immunohistochemistry revealed that Hsp27, Hsp70 and caveolin-1 were localized in basal and suprabasal layers of the epidermis. Caveolin-1 was also detected in fibroblasts in the dermal component. Isolation of caveolar fractions from control and CEES-treated mouse keratinocytes from the skin constructs using sucrose density gradient ultracentrifugation showed that Hsp27 and Hsp70 were localized in caveolae. Treatment of keratinocytes with filipin (20 μM) or methyl-β-cyclodextrin (5 mM), two agents which disrupt caveolar structure, markedly suppressed CEES-induced Hsp27 and Hsp70 gene and protein expression. These data indicate that caveolae-mediated regulation of heat shock protein expression is likely to play an important role in the pathophysiology of vesicant-induced skin toxicity. Supported by CA093798, CA132624, ES004738, ES005022, GM034310 and AR055073.

PL 1751 SULFUR MUSTARD VAPORS INDUCE DNA DAMAGE AND APOPTOSIS IN MOUSE SKIN.

L. B. Joseph[1], D. R. Gerecke[1], A. T. Black[1], R. P. Casillas[2], N. D. Heindel[3], P. J. Sinko[1], D. E. Heck[4], D. L. Laskin[1] and J. D. Laskin[5]. *[1]Rutgers University, Piscataway, NJ, [2]Battelle, Columbus, OH, [3]Lehigh University, Bethlehem, PA, [4]New York Medical College, Valhalla, NY and [5]UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Sulfur mustard (SM, bis (2-chloroethyl) sulfide) is a bifunctional alkylating agent that causes dermal inflammation, edema and blistering. To investigate its mode of action, we used a vapor cup model which provides an occlusive environment in which SM vapor is in constant contact with the skin. The backs of SKH-1 hairless male mice were exposed for 6 min to SM or control; skin was analyzed 1, 3, 7, and 14 days post-treatment. At 1 day, SM caused epidermal thickening, stratum corneum shedding, and basal cell karyolysis; macrophages and neutrophils accumulated in the dermis. Cleaved caspase-3, an apoptotic marker, and phosphorylated histone 2AX (γH2AX), a DNA damage marker, were identified in basal cells. Whereas PCNA expression was contiguous in basal cells of control skin, it was discontinuous after SM treatment. Three days after SM exposure, epithelial cell hypertrophy, edema, parakeratosis, and loss of normal epidermal structures were evident. Increased epidermal expression of enzymes generating pro-inflammatory mediators including cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was also noted. After 7 days, microblisters were evident at the dermal/epidermal junction, and expression of keratin-10, an epithelial cell differentiation marker was upregulated in keratinocytes. Trichrome staining revealed increased collagen deposition in the dermis. PCNA expression in the epidermis was associated with hyperplasia, hyperkeratosis, and parakeratosis. By 14 days, cleaved caspase-3, COX-2 and iNOS expression was decreased, while γH2AX was still evident in the stratum spinosum and stratum granulosum. These findings are similar to reports in humans exposed to SM and show that the SM vapor cup model is appropriate for investigating mechanisms of vesicant-induced DNA damage and apoptosis in the skin. Supported by CA132624, CA093798, ES004738, ES005022, GM034310 and AR055073.

PL 1752 MECHANISMS OF VESICANT-INDUCED CYTOTOXICITY IN LUNG EPITHELIAL CELLS.

Y. Wang[1], D. E. Heck[3], D. L. Laskin[2] and J. D. Laskin[1]. *[1]Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, [2]Pharmacology & Toxicology, Rutgers University, Piscataway, NJ and [3]Environmental Health Sciences, New York Medical College, Valhalla, NY.*

Inhalation of vesicants such as sulfur mustard can cause significant damage to the respiratory system including inflammation, upper and lower obstructive disease, and acute respiratory distress syndrome. A major factor contributing to vesicant-induced lung injury is cytotoxicity and oxidative stress. In the present studies, we used nitrogen mustard (NM, mechlorethamine), a bifunctional alkylating agent and model sulfur mustard vesicant, to characterize cytotoxicity and oxidative stress in A549 cells, a human type II lung epithelial cell line. NM was found to cause a concentration-dependent inhibition of A549 cell growth (IC50 = 1 μM). Pretreatment of the cells with 20 μM buthionine [S, R] sulfoximine (BSO) for 6 hr, which depletes glutathione (GSH), was found to enhance NM-induced growth inhibition (IC50 = 0.2 μM). Cell cycle analysis revealed that 27.1 ± 0.7 % of A549 cells were in the S phase, 18.0 ±1.0 in G2M and 53.4 ± 0.5% in GoG1. Twenty-four hr after treatment of the cells with NM (30 μM, 30 min), we observed a S-phase block, 64.6 ± 1.4% of the cells were in S phase, 25.2 ± 1.5% in G2M and 9.3 ± 0.4 in GoG1. Depletion of GSH in cells had no effect on NM-induced cell cycle arrest. NM (30-300 μM) also enhanced the generation of intracellular hydrogen peroxide, as determined by flow cytometry in conjunction with the hydroperoxy-sensitive probe 2',7'-dichlorofluorescein. Western blotting showed that while NM had no effect on expression of the antioxidant enzymes catalase or heme oxygenase-1; it cross-linked superoxide dismutase, forming a modified 32,000 molecular weight homodimeric protein. Taken together, these data indicate that NM induced cytotoxicity in lung epithelial cells is associated with oxidative stress and alterations in antioxidants, processes that can contribute to vesicant-induced tissue injury. Supported by NIH grants ES004738, ES005022 and AR055073.

PL 1753 HOUSEKEEPING GENE EXPRESSION IN NORMAL HUMAN EPIDERMAL KERATINOCYTES IS ALTERED BY SULFUR MUSTARD EXPOSURE.

S. L. Beach, J. F. Dillman and A. L. Ruff. *U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

Sulfur mustard (SM) is a potent alkylating agent that can cause severe pulmonary, ocular, and cutaneous injury. Many of the molecular mechanisms involved in the response to SM exposure have yet to be fully elucidated, and knowledge of these mechanisms is necessary for the development of therapeutics for this injury. For many molecular assays, it is important to normalize data across samples against housekeeping gene expression. Housekeeping genes are those genes that play an important role in general cell function and are constitutively expressed. The long-held belief that their constitutive expression is not affected by experimental conditions has led to their widespread use for data normalization. Studies are emerging, however, which suggest that experimental conditions can affect the expression levels of housekeeping genes. A previous study by our laboratory showed that the mRNA levels of many housekeeping genes changed significantly in rat lung following SM exposure. Following this observation, we sought to determine if housekeeping gene expression at the mRNA and protein level is affected by SM using the in vitro cutaneous model normal human epidermal keratinocytes (NHEK). Cells were exposed to SM and samples were collected over a 24-hour time period. Housekeeping gene expression was then evaluated in SM-exposed samples and unexposed time-matched controls by microarray and Western blot analysis. Our results suggest that the expression of many housekeeping genes is affected by SM exposure, whereas the expression of others is not. If carefully selected, some housekeeping genes may be appropriate for data normalization in SM-exposed samples. Disclaimer: The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

PL 1754 DEVELOPMENT OF A SULFUR MUSTARD NON-HUMAN PRIMATE INHALATION EXPOSURE MODEL.

W. M. Weber, J. D. McDonald, L. F. Blair, M. R. Lehman, D. A. Kracko, C. Cox, M. Duncan, J. Baldwin, J. Seagrave, T. March and G. R. Grotendorst. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

Sulfur mustard (SM) is a chemical threat agent for which its effects have no current treatment. In a wartime environment, the inhalation exposure scenario is the most likely route of administration to a soldier. Several laboratories are working to develop therapeutics to alleviate SM induced damage. FDA guidance defines the requirement of both small and large animal efficacy to gain approval of a new therapeutic. Small animal inhalation models in rats have previously been reported. Recently, we have developed a non-human primate (NHP) inhalation model for sulfur mustard induced lung injury using cynomolgus macaques. Methods have been developed for the generation and characterization of SM atmospheres through an intubated exposure delivery system to maximize the amount of injury in the lower respiratory tract. Male NHP's were exposed to 100 or 150 mg/m3 of SM for 10 minutes (1000 or 1500 mg*min/m3). Animals were monitored and euthanized at predetermined time points between 3 and 180 days post exposure (DPE).
The pathology induced by the SM inhalation was similar to that in our F344 rat model of SM inhalation injury. Epithelial necrosis and sloughing is apparent along the length of the airway. By 28 DPE there is some regeneration of the airway epithelium but an absence of differentiated airway epithelial cells. Submucosal fibrotic nodules are present and there is an appearance of Collagen V (Col V) leading to the development of auto-antibodies to Col V which are detected in the NHP serum at 28 DPE. This strongly indicates an immunologically based pathological mechanism is being established. The occurrence of the rare pulmonary disorder in human victims of SM exposure and our observations in the NHP model suggest that immunomodulatory therapies will be required for successful treatment of SM inhalation injury. This work was supported by grant number U54 NS058185-04 (S1) from the NIN/National Institute of Neurological Disorders and Stroke.

PL 1755 A TOXICOLOGICAL, BIOCHEMICAL, AND PHYSIOLOGICAL ASSESSMENT OF LUNG AND SYSTEMIC INJURY IN RATS EXPOSED TO INHALED SULFUR MUSTARD ACROSS DOSE AND TIME.

D. S. Olivera[1], K. A. Whitten[2], R. K. Kan[3], J. L. Collins[1], J. B. Simons[1], A. M. Rodriguez[1], C. M. Bowens[1], A. M. Witriol[1] and A. M. Sciuto[1]. *[1]Analytical Toxicology, USAMRICD, Gunpowder, MD, [2]Research Support, USAMRICD, Gunpowder, MD and [3]Research, USAMRICD, Gunpowder, MD.*

Sulfur mustard (HD) is a blistering agent (vesicant) that causes severe chemical burns to the skin, eyes, and airways. HD was used as a chemical warfare agent (CWA) in the Iran/Iraq conflict, and more than half of surviving HD-exposed casualties suffer from permanent lung injuries. HD remains a serious threat with no effective antidote. Despite research in the 90 years since HD was developed as a CWA, the mechanisms and timing of the development of these pathologies are poorly defined. To model the mouth-breathing human, rats were intubated and ventilated for 10 min with nebulized HD or vehicle to achieve total 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-ex-

posure, blood chemistry was analyzed, and lungs were subjected to pathologic analysis. These mid-study data show elevated pCO2 and lowered blood pH and pO2, which correlate with upper airway necrosis and measurements of pulmonary function in the 24-48 h after 3 mg/kg HD exposure. The period between 3 and 7 wks presented a significant challenge because approximately 15% of our high exposure group (3 mg/kg) and 10% of our 6-month LCT50 group (2.25 mg/kg) either died suddenly or required withdrawal from the study during this timeframe. Alveolar exudates, edema, and inflammation peaked at 3 wks and correlated with compensatory changes in pulmonary function and/or respiratory distress. Animals that demonstrated respiratory distress at 3 wks were more likely to die later in the study. Importantly, this indicates that 3-7 wks post-exposure may be a crucial window in the progression of HD inhalation injury, and that therapeutic intervention prior to and concurrent with this time-point may offer the best approach. This study provides the first long-term examination of HD-induced lung injury and systemic effects.

PL 1756 THERAPEUTIC EFFICACY OF CATALYTIC ANTIOXIDANT AEOL 10150 IN ATTENUATING SULFUR MUSTARD ANALOG 2-CHLOROETHYL ETHYL SULFIDE-INDUCED SKIN INJURY.

N. Tewari-Singh[1], A. K. Jain[1], S. Inturi[1], M. Gu[1], C. Agarwal[1], B. J. Day[2], C. W. White[3] and R. Agarwal[1]. *[1]Pharmaceutical Sciences, University of Colorado Denver, Aurora, Co., [2]Medicine, National Jewish Health, Denver, CO and [3]Pediatrics, National Jewish Health, Denver, CO.*

Sulfur mustard (SM), a bifunctional alkylating chemical warfare agent, inflicts devastating injury with delayed vesication of the skin. With the goal to elucidate mechanism/s of SM-caused skin injury as well as to screen for therapeutics, our recent studies with SM analog 2-chloroethyl ethyl sulfide (CEES) have established CEES-induced injury biomarkers in skin epidermal cells and SKH-1 hairless mouse. These studies in SKH-1 hairless mouse skin with CEES and other published reports with SM and CEES have indicated a role for oxidative stress in skin injury caused by these agents. Accordingly, employing our CEES-induced skin injury model, here we determined if treatment with the metalloporphyrin catalytic antioxidant AEOL 10150 could attenuate CEES-induced skin injury. AEOL 10150 treatment of mouse epidermal JB6 cells 1 h post 0.25 mM CEES resulted in over 50% ($p<0.05$) reversal of CEES-induced decrease in cell viability, DNA synthesis inhibition and DNA damage. Similar treatment in human epidermal HaCaT cells also showed significant ($p<0.05$) reversal in CEES-caused decrease in DNA synthesis. Animal efficacy studies were carried out with AEOL 10150, wherein SKH-1 hairless mice were treated with topical formulation (TF) and/or subcutaneously (SC) with 5 mg/kg AEOL 1 and every 4 h after 4 mg/mouse CEES exposure for up to 12 h. Treatment with both TF and SC resulted in over 50% ($p<0.05$) reversal in CEES-caused increase in both skin bi-fold thickness and myeloperoxidase (MPO) activity. Studies are currently underway to further determine the rescue effects of AEOL 10150 employing other established CEES-induced inflammatory and oxidative stress biomarkers from our studies. Nevertheless, results from the current study demonstrate promising therapeutic efficacy of AEOL 10150 in rescue from SM-caused skin injury, warranting further investigation and optimization of its treatment potential.

PL 1757 NANOSILVER MOVEMENT THROUGH BIOLOGICAL BARRIERS RELATES TO PHYSICOCHEMICAL PROPERTIES.

B. Veronesi[1], N. Nazo[1], A. Tennant[1], S. Simmons[1], B. Baruwati[2] and R. Varma[2]. *[1]NHEERL-ISTD, U.S. EPA, Research Triangle Park, NC and [2]NRMRL-STD, U.S. EPA, Cincinnati, OH.*

Linking the physicochemical (PC) properties of engineered nanomaterials (NM) to their biological activity is critical for identifying their (toxic) mode of action, and developing appropriate and effective risk assessment guidelines. Particle surface charge (zeta potential), surface coating and "redox" activity are PC properties thought to influence NM uptake and movement (i.e., translocation) through cellular barriers. Nanosilver (nanoAg) is used in water purification, antiseptics, biocides, medical devices and a variety of consumer and manufactured products. Efforts to reduce its environmental burden and enhance its bioavailability favor "green chemistry" synthesis and the surface coating of particles with benign materials. Since ingestion is a dominant route of exposure, studies were conducted to examine how such PC properties affected nanoAg movement across in vitro models of biological barrier cells. Monolayers of human intestinal (Caco-2) or rat blood brain barrier (RBE4) cells were exposed (3.12-6.25 ppm) to commercially available, capped (citrate or PVP) or uncapped nanoAg particles (10nm and 70 nm). Another sample of nanoAg was synthesized, using a borohydride reduction method, and tested as uncoated particles (50-75 nm) or those coated with glutathione (GSH) or green tea (GT). Microscopy (confocal, TEM) indicated that nanoAg particles translocated barrier cells rapidly (<15 min) and without disrupting their tight junctions. Changes in transcellular electrical resistance (TERS) suggested that surface coatings (citrate>PVP; GSH=GT), an electronegative zeta potential and low aggregate size were PC properties that enhanced nanoAg's cellular movement. Future studies will use high through put techniques to confirm these relationships and will also examine how different surface coatings of nanoAg particles affect their cellular uptake, retention and clearance. (This abstract has been reviewed by NHEERL and does not necessarily reflects EPA policy)

PL 1758 TOXICITY, CELLULAR UPTAKE, AND DIFFERENTIATION OF HUMAN ADULT STEM CELLS EXPOSED TO SILVER NANOPARTICLES.

M. E. Samberg[1, 2], E. G. Loboa[2] and N. A. Monteiro-Riviere[1, 2]. *[1]Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC and [2]Joint Department of Biomedical Engineering at North Carolina State University and UNC-Chapel-Hill, North Carolina State University, Raleigh, NC.*

Orthopedic implantation of tissue engineered bone grafts are becoming a viable alternative to autografts and allografts. Grafts are grown by seeding adult, patient-specific adipose-derived mesenchymal stem cells (hASCs) onto a degradable scaffold and chemically inducing the cells down their osteogenic lineage. A limitation of orthopedic implants pertains to surgical site infections, which result in graft failure and secondary surgery. Silver nanoparticles (Ag-nps) have antibacterial properties that could be incorporated into tissue engineered bone grafts as a preventative measure against surgical site infections. The purpose of this study was to assess the toxicity, cellular uptake, and effect on differentiation of Ag-nps in hASCs. The hASCs were isolated from adipose tissue from elective plastic surgery procedures and either cultured to maintain their self-renewal in growth medium or chemically induced down their adipogenic or osteogenic pathways. During culture, hASCs were exposed to either a single or 7-day repetitive dose of 0.1, 1.0, and 10.0μg/ml of 20nm spherical Ag-nps. Following exposure, toxicity was assessed using alamarBlue viability assay, which showed a significant decrease ($p<0.05$) in viability for both single and repetitive exposures for both osteogenic and adipogenic cells at 1.0μg/ml but not for self-renewing cells. Differentiation of hASCs was examined by Oil red-O (lipid) and alizarin red-S (calcium) and showed no change in differentiation following exposures at any concentration for each of the osteogenic, adipogenic, or self-renewal cells. Transmission electron microscopy was utilized to detect ultrastructural changes and/or differences in uptake of Ag-nps by any of the cells; all cell types were shown to take up the Ag-nps without causing ultrastructural alterations after exposure to all concentrations of Ag-nps. (Supported by US-AFOSR FA 9950-08-1-0182)

PL 1759 PULMONARY TOXICITY FOLLOWING INTRATRACHEAL INSTILLATION OF DISPERSED SILVER NANOPARTICLES IN RATS.

J. R. Roberts[1], R. S. Chapman[1], S. Young[1], A. Kenyon[1], D. Schwegler-Berry[1], A. B. Stefaniak[2], B. T. Chen[1] and J. M. Antonini[1]. *[1]HELD, NIOSH, Morgantown, WV and [2]DRDS, NIOSH, Morgantown, WV.*

Silver nanoparticles (Ag NPs) are emerging as one of the fastest growing categories of manufactured materials in the nanotechnology industry, which may lead to an increased risk of worker exposure to this nanomaterial. The goal of these studies was to characterize pulmonary toxicity of Ag NPs using an in vivo model. Ag NPs were 20 nm in diameter with a polyvinylpovidone coating (PVP, 0.3% wt) (NanoAmor, Inc.). Specific surface area was measured to be 7.54 ± 0.103 m^2/g. Ag NPs were suspended in a dispersion medium (DM, phosphate-buffered saline + 0.6 mg/ml rat serum albumin + 0.01 mg/ml dipalmitoyl phosphocholine) and sonicated. Aggregate size was evaluated in DM using dynamic light scattering and average agglomerates were ~150 nm. On day 0, Sprague-Dawley rats were intratracheally-instilled with Ag NPs in DM at doses of 9.35, 37.6, 112, 224, or 449 μg, SiO_2 at a dose of 449 μg (positive control), or DM alone (vehicle control). Rats were humanely sacrificed 1, 7, 14, and 28 days post-exposure, the right lung was lavaged, and lung-associated lymph nodes (LALN) were removed for analysis. Lavage fluid parameters (lactate dehydrogenase and albumin), cellular influx into the lung (macrophage, neutrophil, and lymphocytes), and LALN cell number and phenotype were evaluated to assess lung injury, inflammation, and immune response. Indices of lung injury and inflammation were significantly elevated in a dose-dependent manner at days 1 and 7, which exceeded the positive control at the highest dose. By day 14, only the rats exposed to 449 μg Ag NP dose had significantly elevated parameters similar to that of the positive control which persisted out to day

28. There was a dose-dependent increase in LALN cell number beginning at day 7 and continuing in the 3 highest doses out to day 28. There was also a dose-dependent increase in lymphocytes in the lavage fluid. Collectively, these data demonstrate the Ag NPs have the capacity to induce pulmonary injury and inflammation, as well as alter immune responses in the lung.

PL 1760 OPTIMIZATION OF HIGH-THROUGHPUT NANOMATERIAL DEVELOPMENTAL TOXICITY TESTING IN ZEBRAFISH EMBRYOS.

A. Wang[1], C. Matson[2], S. Frady[1], M. Arnold[2], S. Padilla[3], R. T. Di Giulio[2] and K. Houck[1]. [1]*National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC,* [2]*Center for the Environmental Implications of NanoTechnology, Duke University Durham, NC and* [3]*ISTD, NHEERL, U.S. EPA, Research Triangle Park, NC .*

Nanomaterial (NM) developmental toxicities are largely unknown. With an extensive variety of NMs available, high-throughput screening methods may be of value for initial characterization of potential hazard. We optimized a zebrafish embryo test as an in vivo high-throughput assay for NM developmental toxicity by assessing 5 zebrafish embryo rearing solutions for their effects on NM aggregation as well as embryo development. We compared the aggregation of NM in 5 solutions [10% Hank's, Danieau, 60 mg/L artificial seawater (ASW), full strength (1X) and 10% EPA moderately hard reconstituted water (MHRW)] by dynamic light scattering analysis. Silver nanoparticles (nano-Ag) coated with citrate-, polyvinylpyrrolidone (PVP)-, or gum arabic (GA) were suspended in these 5 solutions as well as deionized water (ddH_2O) for 24 h. All nano-Ag aggregate sizes in both MHRWs were similar to that in ddH_2O. GA-coated nano-Ag aggregates were largest in Hank's, followed by Danieau, and distantly by ASW. PVP-coated 10 nm nano-Ag and citrate-coated nano-Ag aggregate sizes were slightly larger in Hank's than in ddH_2O. PVP-coated 25 nm nano-Ag sizes were similar in all 5 solutions and ddH_2O. Overall, the aggregates were largest in Hank's, followed by Danieau, ASW, and smallest in MHRWs and ddH_2O. This confirms that aggregation is generally more severe in ion-rich solutions. We reared zebrafish embryos from days 0-6 post-fertilization in these 5 solutions without NM, and used death, malformations, non-hatch and days to hatching as health endpoints. All solutions had the same death and malformation scores. Compared to Hanks, all solutions, except 1X MHRW, delayed hatching. 1X MHRW had fewer non-hatch than 10% MHRW, although not significant. 1X MHRW appears to be optimal for NM testing in zebrafish embryos for being ideal for embryo development and least likely to promote NM aggregation. *This abstract does not necessarily reflect U.S. EPA policy.*

PL 1761 TOXICITY OF INTRANASALLY-ADMINISTERED SILVER NANOPARTICLES.

M. Genter[1], N. C. Newman[1], H. G. Shertzer[1], S. F. Ali[2], S. M. Hussain[3] and B. Bolon[4]. [1]*Environmental Health, University of Cincinnati, Cincinnati, OH,* [2]*Neurochemistry Lab., Division of Neurotoxicology, NCTR/U.S. FDA, Jefferson, AR,* [3]*Applied Biotechnology Branch, AFRL, Wright-Patterson Air Force Base, Dayton, OH and* [4]*GEMpath, Longmont, CO.*

Silver nanoparticles (SNP) are present in numerous consumer and industrial products, due in part to their anti-bacterial properties. Previous *in vitro* studies showed that SNP can adversely affect the blood brain barrier and up-regulate inflammatory cytokines. When injected i.p., 25 nm SNP altered expression of inflammatory and oxidative stress genes in several brain regions. Given current interest in disinfectant sprays containing SNP, we used a more physiologically-relevant route of exposure, intranasal instillation, to examine toxicity endpoints in mice. SNP (25-nm diameter, dispersed in sterile ultrapure water) were intranasally instilled in anesthetized male C57BL/6J mice (100 or 500 mg/kg b.w.). Endpoints measured 1 or 7 days after treatment included histopathology, MAC-3 immunohistochemistry (macrophage marker), reduced glutathione (GSH) levels, and gene expression changes. Body weight and general behavior/activity of treated mice were not affected at either dose or time point. Aggregated SNPs were present in the nasal cavity and associated with epithelial erosions, inflammatory cell infiltrate, and exudates at both doses and time points. One day after exposure, aggregated SNP were visible in spleen, associated with loss of cellularity in the red pulp, and co-localizing with MAC-3 immunoreactivity. Occasional particle aggregates were seen in the lung and renal medulla, with no histopathological changes in structure. Brain and liver histological sections were within normal limits at both doses and time points. GSH, an indicator of oxidative stress, was elevated in blood and nasal epithelia, but not different from control in other tissues. qRT-PCR showed elevated expression of *Kim1*, a marker of kidney damage, indicating that kidney, as well as nasal epithelia and spleen, may be the most significantly impacted tissues following intranasal exposure to 25 nm SNP. Supported by P30-ES06096 and T32-ES010957.

PL 1762 RECOVERY FROM SILVER NANOPARTICLE EXPOSURE-INDUCED LUNG FUNCTION CHANGES IN SPRAGUE-DAWLEY RATS.

I. Yu[1], J. Sung[2], J. Ji[3], K. Song[2], J. Lee[1], J. Lee[4], J. Park[5], H. Park[6], C. Kang[1] and K. Ahn[7]. [1]*Hoseo University, Asan, Republic of Korea,* [2]*Korea Conformity Laboratories, Incheon, Republic of Korea,* [3]*Samung Electronics, Ltd., Suwon, Republic of Korea,* [4]*Occupational Lung Diseases Institute, COMWEL, Ansan, Republic of Korea,* [5]*Chung-Ang University Seoul, Republic of Korea,* [6]*Korea Research Institute of Standards and Science, Daejeon, Republic of Korea and* [7]*Hanyang University Seoul, Republic of Korea.*

In the previous study, the lung function, as indicated by the tidal volume and minute volume, decreased during the 90 days of exposure to silver nanoparticles (AgNPs), and these decreases in the lung function were also accompanied by inflammatory lesions in the lung morphology. Accordingly, this study attempted to investigate the recovery of lung function changes in rats after a 12-week AgNP inhalation exposure to elucidate the process of lung function recovery after cessation of NP exposure. The male and female rats were exposed to silver nanoparticles (14-15 nm diameter) at concentrations of 0.67×10^6 particles/cm^3 (low dose), 1.41×10^6 particles /cm^3 (middle dose), and 3.24×10^6 particles /cm^3 (high dose) for 6 h/day in an inhalation chamber for 12-weeks. Then the rats were allowed to recover from the AgNP exposure. The lung function was measured every week after the daily exposure and after the cessation of exposure, and the animals sacrificed after the 12-week exposure period, and 1 and 3 month after the cessation of exposure. There was exposure related lung function decreases in the male rats during exposure and 3 month recovery period. However, female rats did not show a consistent lung function decrease during exposure period or after the cessation of exposure. Our results suggest that the lung function changes induced by AgNP exposure could be persistent even after recovery period.

PL 1763 CLEARANCE OF ACCUMULATED SILVER AFTER CESSATION OF SILVER NANOPARTICLE (AGNP) EXPOSURE IN TISSUES OF SPRAGUE-DAWLEY RATS.

J. Lee[1], I. Yu[1], Y. Kim[2], K. Song[3], H. Ryu[3], J. Sung[3], M. Kwon[1], J. Park[4], J. Lee[5], H. Park[6], C. Kang[1] and D. Marshak[7]. [1]*Hoseo University, Asan, Republic of Korea,* [2]*KT&G, Daejeon, Republic of Korea,* [3]*Korea Conformity Laboratories, Incheon, Republic of Korea,* [4]*Chung-Ang University Seoul, Republic of Korea,* [5]*Occupational Lung Diseases Institute, COMWEL, Ansan, Republic of Korea,* [6]*Korea Research Institute of Standards and Science, Daejeon, Republic of Korea and* [7]*PerkinElmer, Boston, MA.*

Silver nanoparticls (AgNPs) can be distributed many tissues after oral or inhalation exposure. Clearance of the distributed AgNPs from the tissues is very important to understand the fate of AgNP in vivo. Accordingly, to clarify the clearance of the tissue Ag concentration after the cessation of AgNP oral exposure, Sprague-Dawley rat were assigned to 3 groups: control, low dose (100 mg/kg BW) and high dose (500 mg/kg BW), and administered AgNPs (average diameter 25 nm) by gavage for 28 days. Then the rats were allowed to clear by ceasing the AgNP exposure. The tissue Ag contents in the most tissues such as liver, kidneys, spleen and blood decreased gradually from 1, 2 and 4 months after the cessation of exposure, indicating clearance of AgNPs. In contrast, the silver tissue concentrations in the brain and testis did not decrease rapidly as compared with other tissues even 4 month after the cessation of exposure, suggesting the accumulated AgNP may not be easily transported out from the tissues. Therefore, the tissues with biological barriers such as the blood bran barrier and blood testes barrier may play important role in clearance of AgNPs from the respective tissues.

PL 1764 PREMATURE CELLULAR SENESCENCE-INDUCED BY NANOPARTICLE EXPOSURE: A NOVEL APPROACH TO NANOTOXICITY TESTING.

D. Ellis and S. Hussain. *Air Force Research Laboratory 711 HPW/RHPBA, Wright Patterson Air Force Base, OH.*

Cellular senescence is the natural aging of cells, but premature cellular senescence can be induced by exposure to toxicants. Once a cell enters senescence it will no longer proliferate and it undergoes characteristic changes in phenotype and tissue specific changes in function. In this study we tested the utility of premature cellular senescence as an indicator of nanoparticle toxicity. A549 alveolar epithelial cells were cultured for 2 hours in growth medium containing 10 or 100 nm silver or gold nanoparticles, then allowed up to 6 days of recovery in normal growth medium. Premature cellular senescence was confirmed by an increase in senescence-associated-β-galactosidase (SA-β-Gal) activity, morphological changes, cell cycle arrest, and loss of proliferative potential. The incidence of senescence was cor-

related to oxidative stress by measuring ROS, oxidized proteins, and oxidative DNA damage. Overt toxicity was measured by dye exclusion assay. Both sizes of gold nanoparticles and 100 nm silver nanoparticles did not cause oxidative-stress-related premature cellular senescence in A549 cells. Induction of senescence as indicated by SA-β-Gal activity was a more sensitive indicator of oxidative-stress-related toxicity caused by 10 nm silver nanoparticles than the dye-exclusion assay.

HH 1765 50 YEARS OF "THE PILL": RISK REDUCTION AND DISCOVERY OF BENEFITS BEYOND CONTRACEPTION.

B. Mahadevan[1], K. D. Chadwick[2], R. T. Burkman[3] and B. Tornesi[1]. [1]*Abbott Laboratories, Abbott Park, IL,* [2]*Drug Safety Evaluation, Bristol-Myers Squibb, New Brunswick, NJ,* [3]*Department of Obstetrics and Gynecology, Tufts University School of Medicine, Springfield, MA and* [4]*Worldwide Licensing and Knowledge Management, Merck Research Laboratories, Rahway, NJ.*

Widely regarded as a revolutionary drug in its early years, "the pill" might retrospectively be considered as the first designer or lifestyle drug. Since the introduction of oral contraceptives (OCs) in the 1960s, both health benefits and safety concerns have been attributed to their use. In most instances, the non-contraceptive benefits of OCs outweigh the potential risks. OCs are highly effective, safe, and widely used; approximately 85% of women in the United States will use an OC for an average of five years. However, widespread use of OC formulations by women throughout their reproductive life cycle has given rise to concerns about the effects of OCs on risk factors for coronary heart disease. As with many drug firsts, many lessons can be learned from its development and use. Indeed, "the pill" played a significant role in reshaping pharmacology, social perceptions of medication, and the regulatory process for new drugs during the second half of the 20th century. In addition to the history and side effects/toxicity of OCs, the non-contraceptive health benefits that women experience that expand far beyond pregnancy prevention will be illuminated.

IS 1766 PRECISION-CUT TISSUE SLICES REVISITED: A CLASSICAL METHOD MEETS NEW CHALLENGES.

A. Wolf[1], A. Vickers[2], P. Olinga[3], A. Braun[5], C. Martin[6], G. Groothuis[7] and N. Shangari[4]. [1]*Investigative Toxicology (iTOX), Novartis Institutes of Biomedical Research (NIBR), Basel, Switzerland,* [2]*Investigative Sciences, Allergan Inc., Irvine, CA,* [3]*GUIDE, University of Netherlands, Groningen Area, Netherlands,* [4]*iTOX, NIBR, East Hanover, NJ,* [5]*Department of Immunology, Allergology and Immunotoxicology, Fraunhofer Institute, Hanover, Germany,* [6]*Department of Pharmacology and Toxicology, RWTH Aachen, Aachen, Germany and* [7]*GUIDE, University of Netherlands, Groningen Area, Netherlands.*

The understanding of the underlying mechanisms of a potential drug safety liability and the prediction of a potential risk to man is important from a safety and regulatory perspective. Significant progress in molecular and cellular biology has lead to in vitro models being applied for this purpose. Precision-cut organ slices is an outstanding in vitro model, which maintains the in vivo tissue organization, contains all in vivo relevant cell types, architecture and functional heterogeneity. Historically, organ slice cultures have been applied to study drug metabolism, pharmacology, and compound toxicity. Recently, it has been used to provide information on immune and inflammatory responses, provide insight into pathways of organ injury, assess the involvement of specific cell types in toxicity, and differentiate between species specific responses, thus furthering our understanding in predicting human toxicities and their underlying mechanisms. We will review this latest state-of-the-art use of this technology by providing a forum for the users of precision-cut slices to share their experiences. Our panel of experts will discuss the strengths, opportunities, and limitations of the model to evaluate target organ toxicities. A focal point of our discussions will be the use of slice technology used as a predictive tool in drug development and bridging compound-related safety from different animal species to man. We will provide an overview of the innovative use of toxicity endpoints, functional markers, and demonstrate the use of the model for tailor-made assessment of mechanisms of drug-induced target organ toxicity.

IS 1767 THE APPLICATION OF THE THRESHOLD OF TOXICOLOGICAL CONCERN CONCEPT TO THE PRECLINICAL SAFETY ASSESSMENT OF NON-PHARMACEUTICAL MEDICAL PRODUCTS, INCLUDING MEDICAL DEVICES AND COMBINATION DRUG-DEVICE PRODUCTS.

R. W. Hutchinson[1], R. Brown[2], M. A. Cheeseman[3] and J. Cammack[4]. [1]*Preclinical, Johnson&Johnson: Ethicon, Somerville, NJ,* [2]*CDRH, U.S. FDA, Silver Spring, MD,* [3]*CFSAN, U.S. FDA, Riverdale, MD and* [4]*ISIS Services, San Carlos, CA.*

The Threshold of Toxicological Concern (TTC) is a concept used to estimate safe exposure levels for chemicals for which toxicological data are not available. It is based on chemical structure and known safety data for structurally related chemicals. This value represents a low level of exposure with negligible risk to humans. The process for demonstrating preclinical safety or biocompatibility of medical devices often involves a staged approach starting with a thorough understanding of the chemical composition of the device, then progressing through in vitro and in vivo bioassays and ultimately continuing the risk management process through the lifecycle of the product. The TTC concept is a tool that many risk assessors use to make decisions early in this staged process, and international committees are actively developing standardized methods for applying these techniques. In order to adequately explore this topic, we begin by discussing the history of the TTC concept along with an overview of the areas that this concept has been embraced up to this point. We will provide a description of how tolerable intake levels are set for medical device extractables when an adequate database of information about the extracted chemical is known. In addition, information will be provided on how the TTC concept can be used for extractable chemicals from medical devices for which structural information is known but the toxicological database is insufficient. Finally, the validation of a software package in the context of extractable chemicals known to be relevant to medical devices will be described.

EC 1768 FROM PILOT GRANTS TO HIGH-END JOURNALS: THE SCIENCE OF WRITING.

M. C. Fortin[1] and A. E. Loccisano[2]. [1]*Clinical Research and Occupational Medicine, Environmental and Occupational Health Sciences Institute, Piscataway, NJ and* [2]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Want to learn how to write effective grants and publications, or sharpen your scientific writing skills to communicate better? As toxicologists, it is essential that we be able to articulate new ideas in the form of grants and to disseminate the results of research in the form of scientific publications. Thus effective communication through writing is fundamental therefore it is crucial for early career scientists to learn effective writing skills. Publishing is imperative in academic or non-profit sectors and obtaining sufficient funding is a necessity when establishing a career and reputation. However, most scientists do not receive any formal training in writing and these skills are usually learned by following the style of a mentor or other authors. This issue is particularly important for graduate students, postdoctoral fellows, and other early career scientists who would like to enhance their critical writing skills which are needed for good communication. Our panel of experts will provide the audience with tactics to write promising NIH grant applications, general approaches that enhance the publication success of scientific papers, as well as concrete scientific writing strategies from an author's and reader's standpoint. Attendees will be provided with tips to enhance their skills that will enable more effective communication of both their ideas and their science, from grant proposals to publication.

S 1769 AUTISM: GENETIC, EPIGENETIC, AND ENVIRONMENTAL FACTORS INFLUENCING NEURAL NETWORKS.

I. N. Pessah. *Department of Molecular and Biological Sciences, University of California Davis, Davis, CA.*

Our current knowledge about how genetic, epigenetic, and environmental factors contribute to autism susceptibility is ever changing. From a toxicologist's perspective, the identity of defective genes and signaling pathways linked to autism provide important clues about exposures to environmental chemicals that influence autism susceptibility, severity, and/or treatment outcomes. One fundamental way by which heritable genetic vulnerabilities can amplify the adverse effects triggered by environmental exposures is if both factors (genes x environment) converge to dysregulate the same signaling systems at critical times of development. Thus, we will review current knowledge of genetic contributions to autism risk, and present new

evidence that autism is associated with an appreciably increased level of genomic instability in low copy repeat (LCR) rich intervals of the genome and how environmental factors that affect genomic stability could contribute to the incidence and severity of autism. We will highlight new findings from both a mouse model of Rett Syndrome and human tissues that contribute to our understanding of how developmental exposures to brominated flame retardants (PBDEs) influence DNA methylation and neurobehavioral outcomes relevant to autism. An update will be provided on recent progress in understanding how impairments in neural connectivity contribute to autism including seminal findings of the role of MET polymorphisms play in autism risk and how polyaromatic hydrocarbons found in air pollution might influence MET signaling. Finally, we will review the major translational issues confronting autism research and provide information about current funding opportunities in autism research.

S 1770 COPY NUMBER BURDEN ASSOCIATED WITH AUTISM IN UNSTABLE SEGMENTS OF THE GENOME.

S. B. Selleck[1,2,7], R. L. Johnson[2], J. Balciuniene[7,3], C. M. Conboy[7], T. B. Nauth[2], A. N. Klossner[7], M. Alsagabi[4], A. Alqallaf[4], R. Davis[5], R. Hansen[5], J. Gregg[5], I. Hertz-PIcciotto[5], A. Tewfik[4] and I. N. Pessah[6,5]. [1]*Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA,* [2]*Pediatrics, University of Minnesota, Minneapolis, MN,* [3]*Biology, Temple University, Philadephia, PA,* [4]*Electrical Engineering and Computer Science, University of Minnesota, Minneapolis, MN,* [5]*The Medical Investigations of Neurodevelopmental Disorders Institute, University of California, Davis, Sacramento, CA,* [6]*Department of Molecular Biosciences, School of Veterinary Medicine, UC Davis, Davis, CA and* [7]*Genetics Cell Biology and Development, University of Minnesota, Minneapolis, MN.*

Copy number variants (CNVs) contribute to human differences and to disease susceptibility. CNVs are not uniformly distributed throughout the genome, but are localized in regions rich with segmental duplications (SD) or low copy repeats. Whole genome studies have identified large (>100kb) and rare (<1%) CNVs associated with autism spectrum disorders. To determine if smaller, more common CNVs within relatively unstable segments of the genome contribute to autism (AU) risk, we examined five low copy repeat (LCR)-rich regions where recurrent rearrangements are associated with neurobehavioral disorders. Using high-resolution CGH arrays covering LCRs and surrounding sequences, we evaluated 71 children with AU and 71 typically developing (TD) controls matched for ethnicity and gender. Two of the five genomic regions examined, 7q11 and 22q11-12, displayed significantly increased length of duplicated material in AU. Within a 6Mb interval of 7q11 the preponderance of duplications in AU spans the Williams-Beuren microdeletion segment. Microduplications of this interval were reported in children bearing reciprocal duplication of the Williams deletion interval, 35% of these were diagnosed with AU. Among all 7q11 microduplications reported, expressive language deficits are the prevalent phenotype. Within 22q11-13 the AU group showed very different patterns of copy number change in an interval that includes the Velocardiofacial DiGeorge (VCFG) critical region, with increased frequency of duplications and a paucity of deletions compared to TD. AU-associated duplications and deletions within the VCFG critical region have been previously reported and our findings from finely-tiled arrays suggest imbalances in copy number change for this interval may provide an AU-associated signature. Our findings suggest that copy number alteration in SD-rich intervals may be associated with AU and suggests that relatively common variants in these relatively unexplored regions could provide some discriminatory power. Increased in copy number burden in AU may derive from genetic susceptibility and environmental causes. Environmental factors that affect genomic stability could contribute to the incidence of autism that has, at its foundation, a genetic basis.

S 1771 EPIGENETIC INTERACTION BETWEEN PERINATAL PBDE EXPOSURE AND *MECP2*^{308} MUTATION THROUGH X CHROMOSOME INACTIVATION.

J. LaSalle[1], R. O. Vallero[1], M. Golub[3], J. K. Suarez[1], R. Woods[1], T. Ta[4], A. George[1], P. J. Kostyniak[5], I. N. Pessah[2] and R. Berman[4]. [1]*Microbiology and Immunology, UC Davis School of Medicine, Davis, CA,* [2]*Molecular Biosciences, UC Davis School of Veterinary Medicine, Davis, CA,* [3]*Environmental Toxicology, UC Davis School of Medicine, Davis, CA,* [4]*Neurological Surgery, UC Davis School of Medicine, Davis, CA and* [5]*Toxicology Research, State Univeristy of New York, Buffalo, NY.*

Epigenetic mechanisms act at the interface of genetic and environmental risk factors. Autism has several phenotypic features in common with neurodevelopmental disorders with known altered epigenetic pathways. Rett syndrome (RTT) is an X-linked pervasive developmental disorder caused by mutations in MECP2, which encodes methyl-CpG-binding protein 2 (MeCP2). A mouse model with a truncation of MeCP2 (Mecp2308) has described social behavioral defects and thus is a useful mouse model for testing hypotheses of gene x environment interactions. To test potential genetic, epigenetic, and environmental interactions by perinatal persistent low-dose exposure to the organic pollutant BDE-47, commonly found in human tissues, in an animal model with genetic risk for social and cognitive deficits. Mecp2308/+ dams bred to wild-type C57Bl6/J males were exposed daily to BDE-47 (0, 0.03, or 0.1 mg/kg/day) for 10 weeks (4 weeks pre-mating, 3 weeks in utero, 3 weeks lactation). The 0.1 mg/kg/day BDE-47 exposure negatively impacted fertility and litter survival specifically in Mecp2-mutant but not wildtype C57Bl6/J mice, suggesting an increased genetic susceptibility of Mecp2-mutant mice to BDE-47 in reproductive success. In a litter-based design, pups from dams exposed to BDE-47 (0.03 mg/kg/day) showed behavioral effects in early pre-weaning developmental tests, including impaired sensory neurodevelopment in the Wahlston test battery and ultrasonic vocalizations. In contrast, Mecp2 genotype effects were predominant in juvenile and adult tests showing significant defects in social behaviors, activity and spatial learning. Significant BDE-47/Mecp2 interaction effects were compounding for adult spatial learning, specifically in the heterozygous females. Preliminary results indicate that low dose perinatal BDE-47 exposure results in skewed X chromosome inactivation in Mecp2308/+ offspring. These results suggest that BDE-47, a flame retardant commonly identified in maternal and infant tissues, is a more potent developmental neurotoxicity in a mouse model of RTT.

S 1772 PATHOGENESIS OF CIRCUIT DEVELOPMENT IN THE AUTISMS: EMERGENCE OF THE GENE-ENVIRONMENT AND THE DEVELOPMENTAL NEUROBIOLOGY INTERFACE.

P. Levitt. *Zilkha Neurogenetic Institute, Keck School of Medicine of University of Southern California, Los Angeles, CA.* Sponsor: I. Pessah.

Connecting pathophysiological risk factors for autism spectrum disorder (ASD) requires links between genetic findings with regulation of developing circuits that mediate behaviors most impacted in the disorder. Convergent human genetic and imaging data, and neurodevelopmental studies in animals provide strong evidence for disorder risk through faulty hepatocyte growth factor (HGF) activation of the Met receptor tyrosine kinase. We have worked collaboratively with a number of laboratories to establish a multidimensional approach to the study of ASD risk in the context of understanding disconnection of relevant circuits. ASD risk alleles in the 5' regulatory region of MET has been reported in five independent studies. Gene and protein expression during development is highly regulated during the period of maximal synaptogenesis in the neocortex and limbic system. Studies with Darryl Hood (Meharry) tested the hypothesis that transcriptional regulation of Met is disrupted by polyaromatic hydrocarbons (PAHs). HEK cells transfected with Luciferase reporter constructs containing the MET promoter were tested for their responses to benzo(a)pyrene (BAP), a PAH to which humans are commonly exposed. Cells exposed to B(a)P showed concentration-dependent reduction in MET transcription (5-fold reduction at 500nM B(a)P). Deletion of the Met gene in mice results in alterations of cortical neuron dendrites and spine head volume. With Gordon Shepherd (Northwestern), we obtained electrophysiological data that correlates with the morphological changes, supporting the current ASD model of local cortical hyperconnectivity and long-range underconnectivity. Protein expression in the developing primate brain (with David Amaral, UC Davis) reveals conserved expression with the mouse in developing subcortical limbic structures, but restricted to neocortical circuits that are involved in the processing of emotional visual information. In collaboration with Susan Bookheimer (UCLA), neuroimaging data show striking correlations between the major MET risk allele and under-activation of circuits involved in processing emotion in faces in neurotypical and ASD subjects. These datasets indicate that Met signaling is necessary for the appropriate wiring of forebrain circuits with particular relevance to social and emotional dimensions of behavior that are disrupted in ASD. Future studies will investigate if down regulation of MET transcription recapitulates abnormal cortical networks in the MET knockout model.

S 1773 AUTISM AND THE ENVIRONMENT: CHALLENGES AND OPPORTUNITIES FOR ADVANCING THE SCIENCE.

C. Lawler. *Division of Extramural Research and Training, NIEHS, Research Triangle Park, NC.* Sponsor: I. Pessah.

In response to the urgent public health significance of autism spectrum disorders (ASDs), research efforts in this field have increased markedly over the past five years. Important advances have been made in some areas, including ASD surveillance and genetics, yet the role of the environment remains poorly understood. There is an urgent need for more environmental health scientists to bring their expertise to bear on this problem. Clues emerging from clinical and genetic studies in autism point to perturbation of specific pathways (e.g., immune, synaptic home-

ostasis), but work is needed to translate these findings to cell and animal models. Few exposures have been studied with sufficient systematic rigor to assess ASD risk from the universe of potential exposures. Traditional epidemiologic clues that derive from variation in time and space are elusive in the case of ASD because secular changes in awareness, diagnosis and service provision can obscure real increases in prevalence. Improved infrastructure is needed to support reliable prevalence estimation in many other countries and enable comparison of prevalence in populations with varying exposures. Most of the available evidence supports prenatal origins of ASD, yet measuring exposures during pregnancy requires costly prospective pregnancy cohorts or careful retrospective assessments in other study designs. The collection of environmental data, when it occurs, is not standardized in ways that allow pooling across studies. This is in contrast to the autism genetics field, where combining of data sets is accomplished routinely. Rare structural variants (copy number variation) have been identified as playing key roles in autism causation, yet how and whether environmental exposures contribute to CNVs are unknown. Results from ASD genome wide association studies of normal genetic variation have been inconclusive. This may reflect a failure to consider gene environment interplay. An appreciation of epigenetics as an interface between environment and genes is emerging, yet is only slowly being applied to address questions of autism etiology. In summary, there are many exciting translational challenges in autism research that could benefit from greater involvement of environmental health scientists.

S 1774 GENE-ENVIRONMENT DISEASE INTERACTIONS IN FISH MODELS OF HUMAN DISEASE.

M. J. Carvan[1], J. Postlethwait[2], M. Hahn[3], S. Kullman[4] and J. Bronstein[5]. [1]*Great Lakes WATER Institute, Milwaukee, WI,* [2]*University of Oregon, Eugene, OR,* [3]*Woods Hole Oceanographic Institution, Woods Hole, MA,* [4]*North Carolina State University, Raleigh, NC and* [5]*University of California Los Angeles, Los Angeles, CA.*

Use of alternative species for toxicity testing is a fast growing field in toxicology and provides many distinct advantages as an adjunct to mammalian testing. Thus, we will hightlight the use of small aquarium fish as models to investigate mechanistic toxicity in relation to environment and disease etiology. It is clear that much insight into human health and safety can be gained through research involving various aquatic species. Much effort has been put into using fish species as translational models for detailed mechanistic investigation in cancer, aging, tissue regeneration, genetic diseases and disorders, and stem cell biology. These findings emphasize that most cellular, molecular, and biochemical processes are well conserved across vertebrate groups. The use of aquatic organisms as models in biomedical research has a rich and extensive history. Their research value is widely appreciated, comprising a taxonomically and environmentally diverse group, providing researchers with opportunities to investigate a wide range of systems and processes. New contributions to the toxicology literature reflect in part the strategic use of available resources and chronicle the advantages inherent when these models are coupled to modern experimental approaches in human disease. We will effectively communicate the current state of research with small aquarium fish models in mechanistic toxicology as it relates to molecular approaches addressing gene-environment and environmental-disease interactions. Focus areas will include 'omic approaches, role of gene duplicates and polymorphic sequences in toxicity, and association of genetic and environment factors in disease susceptibility. In conclustion, we will provide a forum for investigators to exchange scientific information and encourage enhancement of the utility of aquatic models for studies of human disease.

S 1775 SOX9, TCDD, GAR, AND DUPLICATE GENE EVOLUTION.

J. H. Postlethwait, A. Amores and Y. Yan. *University of Oregon, Eugene, OR.* Sponsor: S. Kullman.

Zebrafish embryos treated with the dioxin defoliant TCDD mimic the craniofacial phenotype of sox9b knockdown [1] and sox9b, but not sox9a, is one of the most strongly suppressed genes in TCDD treated embryos or regenerating fins [2, 3]. Sox9b mediates TCDD-induced craniofacial malformations [3] and the aryl hydrocarbon receptor (AHR) repressor AHRRB is necessary for full constitutive sox9b expression [4]. Furthermore, TCDD inhibits maturation of ovarian follicles [5], a site of sox9b expression [6]. Teleosts have duplicates of several genes in this network (sox9a/b, ahr1a/b, and ahrra/b [6, 7]) arising from the teleost genome duplication (TGD) [8], which creates opportunities for study due to the partitioning of ancient subfunctions [9]. To explore subfunction partitioning in Sox9 genes, we analyzed regulatory elements in spotted gar (Lepisosteus oculatus), which represents the unduplicated teleost sister lineage [10]. We made a gar fosmid library, sequenced sox9 fosmids, and identified conserved non-coding elements (CNEs) shared with teleosts, many not shared with mammals. Some CNEs were only in sox9a, others only in sox9b, and some in both. To understand gar CNE function, we tested their activity in transgenic zebrafish. Results showed that gar CNEs drive reporter expression consistent with Sox9 activity. We conclude that duplicated genes can evolve by partitioning ancestral regulatory elements. The role of sox9a and sox9b in TCDD toxicity could be explained in part by partitioning, and a similar process may have occurred in ahr1 and ahrr duplicates. 1. Prasch, A.L., et al., Toxicol Sci, 2003. 76,138-50. 2. Andreasen, E.A., et al., Toxicol Sci, 2006. 92,254-69. 3. Xiong, K.M., et al. Mol Pharmacol, 2008. 74,1544-53. 4. Jenny, M.J., et al., Toxicol Sci, 2009. 110,426-41. 5. Heiden, T.C., et al., Reprod Toxicol, 2008. 25,47-57. 6. Chiang, E.F., et al., Dev Biol, 2001. 231,149-63. 7. Hahn, M.E., et al., J Exp Zool A Comp Exp Biol, 2006. 305,693-706. 8. Amores, A., et al., Science, 1998. 282,1711-4. 9. Postlethwait, J., et al., Trends Genet, 2004. 20,481-90. 10. Inoue, J.G., et al., Mol Phylogenet Evol, 2003. 26,110-20.

S 1776 GENE-ENVIRONMENT INTERACTIONS AND DIOXIN SENSITIVITY IN NATURAL AND LABORATORY POPULATIONS OF FISH.

M. E. Hahn[1], S. I. Karchner[1], M. J. Jenny[3, 1], D. G. Franks[1], A. M. Reitzel[1], A. R. Timme-Laragy[1], N. Aluru[1], D. E. Nacci[2] and M. F. Oleksiak[4]. [1]*Biology, Woods Hole Oceanographic Inst., Woods Hole, MA,* [2]*NHEERL, U.S. EPA, Narragansett, RI,* [3]*Biological Sciences, University of Alabama, Tuscaloosa, AL and* [4]*RSMAS, University of Miami, Miami, FL.*

Differential susceptibility to effects of toxicants occurs among species, populations, and individuals, but the underlying molecular mechanisms are not well understood. We are using small fish models to study gene-environment interactions that control the susceptibility of embryos to dioxin-like compounds (DLCs). A population of Atlantic killifish (*Fundulus heteroclitus*) that has evolved resistance to non-*ortho*-substituted PCBs is being investigated to understand the mechanisms underlying the resistant phenotype. Analysis of gene expression profiles by microarray and deep sequencing suggest a genome-wide loss of responsiveness to AHR agonists. The relative roles of aryl hydrocarbon receptor (AHR) duplicates (paralogs) and polymorphic variants of AHR1, AHR2, and AHR repressor (AHRR) in controlling the sensitivity to dioxin toxicity are also being investigated. Fundulus AHR loci are highly polymorphic and display gene- and site-specific patterns of haplotype diversity that are not necessarily associated with functional differences. Dioxin-resistant fish have altered sensitivity to other environmental stressors, such as hypoxia and oxidative stress. Complementary experiments in zebrafish (*Danio rerio*) are testing hypotheses concerning subfunction partitioning in AHR and AHRR paralogs and their roles in regulating embryo sensitivity to DLCs. Results obtained using morpholino anti-sense oligonucleotides suggest that AHRRa and AHRRb have distinct roles in regulating endogenous functions of AHRs and the response to AHR agonists. Zinc-finger nuclease (ZFN) technology is being used for targeted disruption of AHR and AHRR genes in killifish and zebrafish. Together, complementary studies in these two fish models are contributing to an understanding of mechanisms controlling chemical sensitivity during development. [P42ES007381, R01ES006272, R01ES016366]

S 1777 ALTERATION OF DEVELOPMENTAL PROGRAMS REGULATING CARTILAGE AND BONE FORMATION IN TCDD TREATED MEDAKA.

S. W. Kullman[1], W. Dong[1] and D. E. HInton[2]. [1]*Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC and* [2]*Nicholas School of the Environment, Duke University, Durham, NC.*

Defective bone and cartilage development, account for a large number of human birth defects annually. Normal skeletogenesis involves cartilage development in early morphogenetic steps. This process consists of a highly coordinated and orchestrated series of events involving: commitment and differentiation of mesenchymal cells to chondrocytes, programmed and structured maturation and eventual replacement of cartilage by bony structures. Recent developmental studies with laboratory model fish demonstrate that exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) results in cartilage and skeletal abnormalities. In this presentation we use embryonic exposures of medaka to TCDD to illustrate modification in developmental programming for both cartilage and bone formation. Here we focus on hypural plate and neural arch formation where both chondrogenesis and osteogenesis are impaired by TCDD exposure. Characterization of respective phenotypes are conducted through high resolution imaging of the hypural plate using conventional staining of bony and cartilaginous structures, epifluorescence of intact transgenic medaka expressing RFP under control of the osteoblast specific osterix promoter and transmission electron microscopy. Defined phenotypes are anchored through gene expression analysis of chondrocyte and osteoblast cell markers. Global

gene expression studies illustrate that dysregulation of key transcription factors governs both chondrogenesis and osteogenesis and that each are impaired by TCDD exposure. These studies are central to our ongoing studies into cellular and molecular mechanisms regulating environmental perturbations in these model organisms.

S 1778 INFLUENCE OF HUMAN GENE VARIANTS ON THE EFFECTS OF DEVELOPMENTAL MEHG EXPOSURE.

M. J. Carvan[1], R. Klingler[1] and J. Topczewski[2]. [1]*School of Freshwater Sciences, Great Lakes WATER Institute, University of Wisconsin-Milwaukee, Milwaukee, WI and* [2]*Children's Memorial Research Center, Northwestern University, Chicago, IL.*

Children prenatally exposed to methylmercury (MeHg) display a range of effects varying from severe cerebral palsy to subtle developmental delays. Many of the more subtle effects of developmental MeHg exposure can be permanent, including behavioral abnormalities, nervous system deficits, and loss of IQ. It is estimated that as many as 15% of American women of childbearing age have total blood mercury concentrations above that associated with neurological deficits in children. Zebrafish suffer neurological deficits (learning and memory, and visual system problems) at exposures similar to those that affect humans and these deficits persist into adulthood and across generations. We are using genetically-modified zebrafish to explore the influence of human polymorphisms in glutathione-related genes on sensitivity to MeHg-induced toxicity and neurological deficits. In this model, zebrafish genes have been disrupted using zinc finger nucleases and the fish express transgenes encoding normal or polymorphic human alleles. Model systems such as this represent a new approach for rapid mechanistic evaluation in a whole organism of human polymorphisms related to environmental disease.

S 1779 INVESTIGATING ENVIRONMENTAL AND GENETIC RISK FACTORS OF PARKINSON'S DISEASE USING ZEBRAFISH.

J. Bronstein, A. Kotagiri, S. Prabhudesai, S. Sinha, S. Li and G. Bitan. *Neurology, University of California Los Angeles School of Medicine, Los Angeles, CA.* Sponsor: M. Carvan.

Parkinson's disease (PD) is a neurodegenerative disorder resulting in loss of a variety of neurons although dopaminergic (DA) neurons appear to be selectively vulnerable. The etiology of PD is not completely understood but likely involves both environmental (e.g. pesticides) and genetic factors. A major goal of our laboratory is to determine if pesticides associated with developing PD are causal and if so, by what mechanisms. We generated a transgenic zebrafish (ZF) that expresses GFP in tyrosine hydoxylase (zTH-GFP) expressing neurons (a marker of DA neurons). A genomic construct was isolated and found to confer both the spacial and temporal expression of endogenous TH. A 2-photon microscope was used to ablate labeled neurons in the diencephalon (DC) of zTH-GFP ZF to determine the phenotype with loss of DA neurons as seen in PD. Ablation of a few TH clusters in the DC resulted in death or altered locomotion. Treatment of zTH-GFP ZF with PD-associated toxins resulted in altered TH neuron morphology and locomotion. For example, treatment with the fungicide ziram (1 nM) resulted in dramatic changes in morphology of TH neurons. In addition to toxins, several lines of evidence have implicated the involvement of α-synuclein (α-syn) in the pathogenesis of PD. We developed a ZF model to study the mechanisms of α-syn toxicity and accelerate drug discovery for mitigating its toxicity. Human α-syn was over-expressed in neurons using a bicistronic construct with DsRed and a self cleaving T2A signal so we could visualize transgene expression without altering α-syn activity. Expression of α-syn resulted in aggregate formation, inhibition of the ubiquitin-proteasome system (UPS), and neuronal cell death. Treatment of α-syn ZF with CLR01, a molecular tweezer that prevents α-syn fibril formation, dramatically improved ZF morphology and survival, neuronal cell death, and UPS activity. These ZF models, and others in development, offer unique opportunities to study environmental and genetic factors implicated in the development of PD.

S 1780 MECHANISMS OF INFLAMMATION IN SKIN CARCINOGENESIS.

L. Mordasky Markell and M. G. Borland. *Center for Molecular Toxicology and Carcinogenesis, Penn State University, University Park, PA.*

The World Health Organization finds that 30% of all human cancers are cancer of the skin, and one in five Americans will develop skin cancer in their lifetime. Furthermore, increased cancer mortality has been linked to a history of non-melanoma skin cancer. The majority of skin cancers arise from chemical contact or exposure to ultraviolet radiation, but a complete understanding of the mechanisms involved in cancer development remain elusive. Chronic irritation and inflammation have also been observed at sites of tumorigenesis; this, coupled with the fact that tumors routinely present with deregulated proliferative signaling pathways, suggests inflammatory signaling may significantly contribute to skin carcinogenesis. Understanding how inflammation alters tumor development and progression could identify novel preventative and therapeutic strategies to treat or reduce skin cancer. Our divergent panel of experts will discuss the mechanisms by which the inflammatory micro-environment alters skin tumor formation and progression, the importance of epidermal homeostasis, the differential roles of key inflammatory cells that contribute to the tumor phenotype, how mouse models can be applied to human relevance, and how this research impacts risk assessment and regulation. Because inflammation has been implicated in other cancers, investigators can apply, exploit, and build upon the discussed strategies and mechanisms for other cancer models. For those investigators interested in the integrative and multi-disciplinary nature of cancer biology, this discussion and collaboration will lay the groundwork to help understand the novel mechanisms of skin carcinogenesis. In conclusion, exploration of this topic will enable us to identify potential targets that can be related to the cause of cancer ultimately striving to decrease the incidence and mortality of this very common worldwide cancer.

S 1781 A SERIES OF AUTOCRINE AND PARACRINE FEEDBACK LOOPS IN INFLAMMATION PATHWAYS ARE REQUIRED FOR TUMOR FORMATION IN SKIN CARCINOGENESIS.

S. H. Yuspa. *National Cancer Institute, National Institutes of Health, Bethesda, MD.* Sponsor: L. Markell.

Constitutively active RAS plays a central role in the development of skin cancer in the classical two stage skin carcinogenesis in mice and in a number of human cancers. Ras mediated tumor formation is commonly associated with upregulation of cytokines and chemokines that mediate an inflammatory response considered relevant to oncogenesis. MyD88-/- is a crucial intermediate in the expression of multiple innate immune responders through signaling from the Toll-like/IL-1R family. Mice ablated for MyD88 or the IL-1R are resistant to topical skin carcinogenesis, and cultured MyD88-/- keratinocytes transduced with an oncogenic ras vector form only a few small tumors in orthotopic grafts. Initiated keratinocytes arising from oncogenic activation of Ras mimic the phenotype of *in vivo* induced skin tumors and are hyperproliferative but also resist signals for induced differentiation and upregulate cytokine, chemokine, and metalloprotease genes that *in vivo* mediate an inflammatory response considered relevant to oncogenesis. Oncogenic Ras transduced MyD88-/- keratinocytes are also hyperproliferative but the differentiation response is intact and pro-inflammatory genes are not upregulated. Using both genetic and pharmacological approaches, we find that in keratinocytes, the differentiation and pro-inflammatory functions mediated by oncogenic ras require the establishment of an autocrine loop through IL-1alpha and its receptor leading to NF-κB activation. In the absence of MyD88 or IL-1R, this loop cannot be established. Further, blocking the IL-1alpha mediated NF-κB activation in ras-transduced wildtype keratinocytes corrects the defect in differentiation response and inhibits proinflammatory gene expression. Collectively, these results demonstrate that Ras activation converts normal keratinocytes to an initiated phenotype through a series of potentially reversible feedback signals that provide therapeutic opportunities through inhibition of IL-1 signaling.

S 1782 IKKα FUNCTION IN SKIN INFLAMMATION AND TURMORIGENESIS.

Y. Hu. *NCI, Frederick, MD.* Sponsor: L. Markell.

IKKα is a tumor suppressor for skin squamous cell carcinomas (SCCs) in human and mice. Ikkα-/- mice develop a malformed and thick skin lacking terminal differentiating keratinocytes. IKKα deletion in keratinocytes causes epidermal hyperplasia and spontaneous skin papillomas and SCCs in adult mice. Reduction of IKKα promotes chemical carcinogens- and UVB-induced skin tumor initiation and progression. We further found that nuclear IKKα represses transcriptional activities of EGF, HB-EGF, AR, and A disintegrin and metalloprotease (ADAMs) genes, thus, IKKα loss upregulates these growth factors, leading to elevated EGFR, Ras, and ERK activities. This elevated loop not only promotes keratinocyte proliferation that represses differentiation, but also crosstalks with inflammation pathways. Reintroduction of IKKα or inactivation of EGFR inhibits IKKα deletion-induced proliferation, various cytokine products, and inflammation during tumorigenesis. Furthermore, IKKα is a subunit of the IKK complex, a central regulator for activation of NF-κB that is important for immune and inflammatory responses. IKKα loss and reduction was found to elevate IKK/NF-κB activity and cy-

tokine products during carcinogenesis. Taken together, these results reveal that IKKα is essential for embryonic skin development and plays a critical role in maintaining skin homeostasis and preventing skin SCCs through regulating skin cell differentiation, proliferation, and inflammation pathways.

S 1783 MODELING THE INFLAMMATORY TO IMMUNOSUPPRESSIVE SWITCH DURING PREMALIGNANT PROGRESSION.

A. Glick and A. Gunderson. *Veterinary and Biomedical Sciences, Penn State University, University Park, PA.* Sponsor: L. Mordasky Markell.

Immunosuppression within the developing tumor microenvironment frequently occurs under conditions of acute or chronic inflammation. Myeloid derived suppressor cells (MDSC), a heterogeneous population of immature myeloid cells (Gr-1+/CD11b+) that are greatly expanded in response to tumor derived factors, play a significant role in generating systemic and localized immunosuppression in both human cancer patients and experimental mouse models. Here we have investigated the role of MDSC formation in skin carcinogenesis using a a bitransgenic model in which oncogenic human c-Ha-RasG12V is induced in the suprabasal layers of the mouse epidermis with the doxycycline regulated Involucrin-tTA driver line. Induction of RasG12V in bitransgenic mice caused rapid epidermal hyperplasia and cutaneous inflammation as expected, which was largely composed of infiltrating neutrophils that formed cytotoxic microabscesses in the suprabasal layers of the epidermis. Antibody mediated depletion of neutrophils concurrent with transgene activation ablated neutrophil infiltration, reduced microabscesses and caused an increase in epidermal thickening. Ras-induced neutrophils were cytotoxic towards neoplastic keratinocytes in vitro. On a Rag1 null background this cytotoxic inflammatory response was suppressed, and restored by adoptive transfer of T cells but not B cells. However, 2 weeks post-Ras, neutrophil microabscesses were replaced by a diffuse single cell infiltration of myeloid cells, coincident with increased systemic levels of Gr-1/CD11b cells that could suppress in vitro proliferation of CD4 and CD8 T cells, decreased neutrophil cytotoxicity in vitro, and decreased cutaneous IFNγ+ T cells. By 2 weeks the hyperplasic epidermis had progressed to focal SCC and keratoacanthomatous-like lesions. Surprisingly markers of infiltrating MDSC and tumor progression and invasion such as MMP9 were significantly reduced on a Rag1 null background, indicating a requirement of the adaptive immune system for generation of the MDSC response and tumor progression.

S 1784 DOES SEX MATTER IN THE DEVELOPMENT OF NON-MELANOMA SKIN CANCER?

T. Oberyszyn[1], N. J. Sullivan[1], K. L. Tober[1], M. A. Bill[2], J. A. Riggenbach[1], J. S. Schick[1] and G. B. Lesinski[2]. [1]*Pathology, The Ohio State University, Columbus, OH and* [2]*Internal Medicine, The Ohio State University, Columbus, OH.* Sponsor: L. Mordasky Markell.

Ultraviolet light B (UVB) is a major environmental carcinogen that has been implicated in the development of nonmelanoma skin cancers (NMSC). These skin tumors are the most common form of cancer in humans, with over 1 million new cases identified in the United States each year. In fact, more Americans will be diagnosed with some form of skin cancer than all other cancers combined. Epidemiological studies have shown that there are sex differences in the development of NMSC, with men being three times as likely to develop squamous cell carcinomas as women. This disparity has been attributed to lifestyle choices. However, recent studies from our laboratory using the Skh-1 mouse model suggest that the pathways by which UV exerts its damaging effects in male and female skin are not the same. While male murine skin exhibited less UVB-induced inflammation compared to female mice, they displayed higher levels of epidermal oxidative DNA damage and lower total antioxidant capacity both prior to and 48 hours following a single UV exposure. Long-term studies showed that when exposed to equal doses of UVB, male Skh-1 mice developed tumors earlier, in greater number and with a more advanced grade than female mice. One major factor thought to contribute to inflammation-induced immune suppression and consequent carcinogenesis is a heterogeneous population of myeloid cells, known as myeloid-derived suppressor cells (MDSC). The Skh-1 model provides a unique opportunity to examine MDSC expansion before and throughout tumor progression. We found that male mice exhibited increased splenic MDSC expansion both following acute as well as chronic UVB exposure. After adjusting for tumor burden, sex was an independent predictor of elevated splenic MDSC expansion, with male tumor-bearing mice having 55% higher splenic MDSC levels. While our model focuses on skin cancer, these findings also have the potential to impact the development of therapeutics for other types of inflammation-associated carcinomas.

S 1785 BIOMARKERS AS INDICATORS OF PHOTOTOXICITY: DO THEY HAVE A ROLE AS PREDICTORS OF PHOTOCARCINOGENESIS?

D. B. Learn[1], P. D. Forbes[2], C. P. Sambuco[1], A. M. Hoberman[1] and S. R. Eldridge[3]. [1]*Charles River Laboratories Preclinical Services, Horsham, PA,* [2]*Toxarus, Inc., Malvern, PA and* [3]*Charles River-Pathology Associates, Frederick, MD.*

Solar UVR exposure causes well-recognized dose- and time-dependent cutaneous DNA damage, rapid-onset erythema (vasodilation) and edema, persistent erythema with inflammatory infiltrate, impaired cutaneous immune function, cell proliferation and hyperplasia. Together, these effects may be considered a "perfect storm" of pre-cancerous signals in skin. Because its delivery can be precisely controlled and distributed, UVR exposure provides an ideal stimulus for modeling neoplastic precursors, including identifying relevant inflammatory markers and exogenous agents that influence these markers and thus the course of UVR photocarcinogenesis. Laboratory and clinical studies show that the effectiveness of a given UVR dose can be modified by many factors, with one example being the influence of pharmaceuticals on experimental photocarcinogenesis. But can biomarkers predict this influence? What constitutes an inflammatory or neoplastic biomarker? Which biological precursors are necessary or sufficient for neoplastic development? Which markers, if any, are predictive of neoplastic enhancement? Unresolved questions temper the understandable urge to replace photocarcinogenesis testing with tests based on measuring biomarker responses. In general, variables that influence inflammation (during or after UVR exposure) tend to have corresponding influences on the rate of photocarcinogenesis. However, the apparent relationship has its limits, may not correlate with anticipated effects and in known cases (e.g., anthracene or porphyrin phototoxicity), erythema is not associated with enhanced photocarcinogenesis, connoting a "false positive". Whether tumor enhancement can occur in the absence of these markers (i.e., a "false negative") is not established. Thus, alternative tests based on biomarkers may function as effective screening tools but are not yet ready to displace the more definitive photocarcinogenesis test for risk identification.

S 1786 NEW INSIGHTS INTO THE NRF2-KEAP1 PATHWAY AND ITS IMPACT ON HUMAN DISEASE.

D. D. Zhang[1] and T. W. Kensler[2]. [1]*University of Arizona, Tucson, AZ and* [2]*University of Pittsburgh, Pittsburgh, PA.*

Damage mediated by reactive electrophilic intermediates can have a profound effect on many cellular functions, and has been implicated in cancer, inflammation, neurodegenerative diseases, cardiovascular diseases, and aging. Eukaryotic cells have evolved anti-oxidant defense mechanisms to neutralize reactive oxygen species (ROS) and maintain cellular redox homeostasis. Eukaryotic cells also express cofactors and enzymes for trapping reactive electrophiles. A key adaptive response system for protection against ROS and electrophilic intermediates is mediated by the transcription factor Nrf2, through the antioxidant responsive element (ARE) sequences in the promoter regions of dozens of cytoprotective genes. Basal levels of Nrf2 remain relatively low, due to its negative regulation by Keap1, which targets Nrf2 for ubiquitination and degradation. However, upon activation, cysteine residues of Keap1 are modified, leading to conformational changes that impair ubiquitination of Nrf2, thus allowing Nrf2 to translocate into the nucleus to activate the expression of its downstream genes. The activation of the Nrf2 pathway is not only important in protecting cells against the deleterious effects caused by carcinogens and environmental toxicants, but, also, Nrf2 protects against drug-induced organ toxicity and damage. Unfortunately, the dark-side of Nrf2 has been revealed indicating that constitutive activation of Nrf2, due to mutations in either Nrf2 or Keap1, creates an environment conducive to cancer cell growth and thus, contributes to chemoresistance. Our knowledge on the Nrf2 pathway has progressed rapidly, and new important insights into its complex regulation have emerged. Thus it is important to highlight the novel mechanisms that activate the Nrf2 pathway, cross-talk between Nrf2 with other essential cell signaling pathways to maintain cellular homeostasis, the protective role of Nrf2 in human diseases, and paradoxically its role as a hostage in cancer. In conclusion, we will explore the essential role of Nrf2 in disease and in a variety of biological processes that regulate the response to environmental exposures.

S 1787 THE NRF2-KEAP1 STRESS RESPONSE PATHWAY: MOLECULAR BASIS OF ADAPTIVE RESPONSES AND PATHOGENESIS.

M. Yamamoto, H. Kurokawa, T. Suzuki, K. Taguchi and H. Motohashi. *Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan.* Sponsor: T. Kensler.

Our bodies must counteract insults originating from the environment. Electrophiles and ROS transcriptionally activate the expression of detoxifying and antioxidant genes through the antioxidant/electrophile responsive element

(ARE/EpRE). Transcription factor Nrf2 is essential for the coordinated induction of cellular defense enzymes through ARE/EpRE. Detailed analysis of the regulatory mechanisms governing Nrf2 activity led to the identification of Keap1 that represses the Nrf2 activity by binding to the N-terminal Neh2 domain. Electrophiles liberate Nrf2 from the repression by Keap1 and provoke the nuclear accumulation of Nrf2, demonstrating that the Nrf2-Keap1 system acts as a sensor for xenobiotic and oxidative stress. The two-site substrate recognition model proposed for the Keap1-Nrf2 system describes the regulation of nuclear accumulation of Nrf2 by a Cul3- Keap1 E3 ubiquitin ligase-dependent mechanism. We have tested this model through structure biology, mouse/zebrafish genetics and human cancer analyses. Keap1 forms homodimer resembling a cherry-bob. The Keap1-DC domain binds to the Neh2 domain of Nrf2. Binding of both high affinity ETGE and low affinity DLG motifs in the Neh2 to two DC domains of the Keap1 homodimer allows correct positioning of the ubiquitin-target lysine residues within α-helix of the Neh2 domain for efficient ubiquitination. Oxidative or electrophilic stress modifies reactive cysteines of Keap1. We surmise that these modifications lead to alteration of the local conformation and result in the Keap1 dysfunction. The Nrf2 inducers can be categorized into several classes based on the interacting cysteine residues, and this observation suggests that multiple sensor functions reside within Keap1. The Nrf2-Keap1 system opens a new avenue to the understanding of the signal transduction and regulatory processes underlying the stress response and cancer progression.

S 1788 NRF2 IN TOXICOLOGY: FROM MONOFUNCTIONAL INDUCERS TO THERAPY.

C. D. Klaassen. *Pharmacology, University Kansas Medical Center, Kansas City, KS.*

In the late 1980s, Paul Talalay pioneered the idea that some inducers had little effect on phase-I enzymes but induced phase-II enzymes. In retrospect, this concept of monofunctional inducers explained many confounding results in toxicology, such as the protective effect of butylated hydroxyanisole against acetaminophen hepatotoxicity and the observation that oltipraz diminished aflatoxin-induced carcinogenicity. In 1990, Cecil Pickett's group reported that induction of glutathione S-transferases (GSTs) was dependent on an antioxidant response element (ARE) found in genes, and in 1997 Yamamoto and colleagues discovered that Nrf2 was the transcription factor necessary for the induction. Thus, many monofunctional inducers are now referred to as Nrf2 activators, which induce genes containing AREs. Our work on Nrf2 began in the late 1980s when Jie Liu came to my laboratory from China with several compounds extracted from Chinese herbal medicines. He tested 16 of these chemicals for hepatoprotective effects and determined one chemical, oleanolic acid, was the most effective. In fact, oleanolic acid protected against nine different hepatotoxicants with seemingly different pathways of producing toxicity. Michael Sporn and his group synthesized many derivatives of oleanolic acid, and eventually 2-cyano-3,12-dioxooleana-1,9-dien-28-imidazolide (CDDO-Im) emerged as the most potent derivative. We now know that Nrf2 activators increase not only the GSTs and other phase-II enzymes, but also glutathione synthesis enzymes, heme oxygenase-1, NAD(P)H:quinone oxidoreductase 1, microsomal epoxide hydrolase, and various enzymes that detoxify reactive oxygen species, all of which cumulatively produce robust anti-oxidant and anti-inflammatory effects. Thus, it is not surprising that activators of the Nrf2 pathway, like oleanolic acid, can protect against the toxicity of many chemicals. It is interesting to note that another derivative of oleanolic acid (CDDO-Me, bardoxolone methyl) is in Phase 2 clinical trials as an activator of the Nrf2 pathway for the treatment of chronic kidney disease in diabetic patients. (NIH DK-081461)

S 1789 NRF2: NOT JUST FOR PHASE 2 ENZYMES ANYMORE.

T. W. Kensler[1, 2]. [1]*Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, PA and* [2]*Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Evaluation of the role of the Nrf2 signaling pathway on cytoprotection has focused predominantly on the regulation of phase 2 enzymes involved in the detoxication of toxins and their reactive intermediates such as glucuronosyl transferases and glutathione transferases. While these enzymes are important modifiers of toxicological responses, recent evidence highlights a much broader role for Nrf2-regulated genes in promoting cell survival. Not only is the detoxication of reactive oxygen and electrophilic metabolites enhanced, but so are mechanisms for the recognition, repair or removal of damaged macromolecules through up-regulation of nucleotide excision repair and proteasome systems. Moreover, a link to facilitated tissue repair has been observed with the observation that Nrf2 regulates the expression of Notch1, a transcription factor influencing stem cell fate. This presentation will discuss the expanding role of the Nrf2 pathway as an adaptive stress response against reactive intermediates and their macromolecular damage, and for promoting tissue repair and regeneration.

S 1790 GAIN OF NRF2 FUNCTION AS A TARGET FOR ANTICANCER THERAPY?

S. Biswal and A. Singh. *Johns Hopkins University, Baltimore, MD.* Sponsor: D. Zhang.

Nuclear factor erythroid-2 related factor 2 (NRF2) is a redox-sensitive transcription factor that positively regulates the expression of genes encoding antioxidants, xenobiotic detoxification enzymes, and drug efflux pumps, and confers cytoprotection against oxidative stress and xenobiotics in normal cells. Kelch-like ECH-associated protein 1 (KEAP1) negatively regulates NRF2 activity by targeting it for proteasomal degradation. We recently reported that NRF2-KEAP1 interactions are deregulated in lung cancer (mostly adenocarcinomas) and prostate cancer, through a variety of complementary mechanisms. Somatic alterations in Keap1 gene were detected in a high percentage of the cancer cell lines and primary lung and prostate tumors. In addition to mutations, we found novel transcriptional and post transcriptional mechanisms of KEAP1 inactivation such as promoter methylation and aberrant splicing. Silencing of Nrf2 expression, by constitutive expression of short hairpin RNA targeting Nrf2 in lung and prostate cancer cells, induced generation of ROS, suppressed tumor growth and resulted in increased sensitivity to chemotherapeutic drug-induced cell death in vitro and in vivo. Gain of NRF2 function in cancer cells also confers radioresistance. Furthermore, KEAP1 inactivation accelerates oncogenic K-RAS-induced lung tumorigenesis whereas loss of NRF2 function suppresses K-RAS driven lung tumor growth in mice models. Preliminary studies have indicated that high NRF2 activity in cancer cells upregulates the glucose flux through central pentose phosphate pathway and tricarboxylic acid cycle leading to increased ribose synthesis, de novo fatty acid synthesis, and energy metabolism, which in turn promotes cellular proliferation and tumorigenesis. This presentation will focus on presenting evidence that gain of NRF2 function in cancer helps tumor cells to exploit the NRF2 dependent cytoprotective and metabolic pathways for maintaining their tumorigenic potential and survival against cytotoxic chemotherapeutic agents and radiation therapy. (This work was supported by NIH grants P50CA058184, P30ES038819, R01CA140492 and FAMRI)

S 1791 GETTING CAUGHT IN THE WEB OF NRF2.

D. D. Zhang. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

During the last couple of years, crosstalk between the Nrf2 pathway and other important pathways has emerged. Our group has identified two separate branches that converge with the Nrf2 pathway, the p53-p21(Cip1/WAF1) pathway and the autophagy pathway. Crosstalk is mediated by the direct interaction between p21 and Nrf2, and Keap1 with p62, respectively. This presentation will consist of two parts. In part 1, I will present molecular and genetic evidence suggesting that the previously suggested antioxidant function of p53 or p21 is mediated through activation of the Nrf2 pathway. Mechanistically, p21 is able to stabilize Nrf2 by competing away Keap1, thus, activating the Nrf2-mediated antioxidant response. Therefore, the interaction between Nrf2 and p21 represents a fine-tuning mechanism between life and death according to the level of stress. In part 2, a novel mechanism of Nrf2 activation by autophagy deficiency through a direct interaction between Keap1 and p62 will be presented. In response to stress, cells can utilize several cellular processes, such as autophagy, a bulk-lysosomal degradation pathway, to mitigate damages and increase the chances of cell survival. Deregulation of autophagy causes upregulation of p62 and the formation of p62-containing aggregates, which are associated with neurodegenerative diseases and cancer. Accumulation of endogenous p62 or ectopic expression of p62 sequesters Keap1 into aggregates, resulting in the inhibition of Keap1-mediated Nrf2 ubiquitination and its subsequent degradation by the proteasome. In contrast, overexpression of mutated p62, which loses its ability to interact with Keap1, had no effect on Nrf2 stability, demonstrating that p62-mediated Nrf2 upregulation is Keap1-dependent. These findings demonstrate that autophagy deficiency activates the Nrf2 pathway in a non-canonical cysteine-independent mechanism.

W 1792 APPROACHES FOR INCORPORATING NON-CHEMICAL STRESSORS INTO CUMULATIVE RISK ASSESSMENTS.

C. V. Rider[1] and J. Simmons[2]. [1]*Toxicology Branch, NIEHS-NTP, Research Triangle Park, NC and* [2]*Pharmacokinetics Branch, ISTD, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC.*

Realistic human exposures are comprised of multiple chemical as well as non-chemical stressors. Non-chemical stressors, such as poor diet and physiological or psychological stress, often elicit adverse effects by targeting the same signaling path-

ways as chemical stressors and, therefore, have the potential to significantly modify our responses to chemical exposures. In recent years, regulatory agencies have emphasized the need for conducting cumulative risk assessments in order to account for real-world, multi-chemical exposure scenarios; however, the incorporation of non-chemical stressors into these cumulative risk assessments represents a novel and complex challenge. Development of a systematic approach for assessing the joint action of chemical and non-chemical stressors is needed in order to prioritize non-chemical stressors for inclusion in cumulative risk assessments, determine the type of data needed to incorporate non-chemical stressors, and develop methods for assessing their contribution to overall risk. We will highlight key issues in order to provide a rational starting place for moving forward. Therefore it is important to put this issue in context, describe data needs, and propose methods for incorporating nonchemical stressors into cumulative risk assessments. Participants represent perspectives from regulatory agencies, industry, non-profit, and academia.

W 1793 CURRENT REGULATORY PERSPECTIVE ON INCORPORATING NON-CHEMICAL STRESSORS INTO CUMULATIVE RISK ASSESSMENTS.

M. M. Mumtaz. *Computational Toxicology and Methods Development Laboratory, ATSDR, Atlanta, GA.*

The global risk assessment community, most recently, is trying to address concerns regarding climate change and a gamut of stressors that could impact human populations and ecological systems. The U.S. Congress has passed several laws that mandate federal agencies to perform cumulative risk assessment (CRA) thus recognizing population vulnerability factors (such as diet, behaviors, genetic traits, economic status) and social characteristics as important issues to be considered in health risk assessment. As practiced, CRA is an analysis, characterization, and possible quantification of the combined risks to human health or the environment from multiple agents or stressors. CRA provides the integrating foundation for linking chemical agents or stressors across space and time. It utilizes a population perspective, incorporating vulnerability factors (i.e., susceptibility/sensitivity and differential exposure, preparedness and ability to recover) into the assessment. To address multiple stressors and complex exposure scenarios, CRA will need to use advanced risk assessment methods: kinetic models to assess target tissue dose; extrapolations of risks across doses, species, and routes; and assessment of multiple effects to account for both primary and secondary adverse health effects. Often, the lack of toxicological data reporting that leads to an inability to identify "common metrics" is the key limiting factor to performing cumulative risk assessment of multiple stressors. A perspective on the concepts/methods of CRA including the integration of stressor/chemical interactions with vulnerability factors will be presented.

W 1794 A CONCEPTUAL FRAMEWORK FOR CHARACTERIZING FACTORS THAT INFLUENCE EXPOSURES TO CHEMICAL AND NON-CHEMICAL STRESSORS.

P. S. Price. *Toxicology & Environmental Research & Consulting, The Dow Chemical Company, Midland, MI.*

All communities are exposed to multiple stressors. Characterizing the magnitude of the stressors and their cumulative impact presents major challenges to the exposure assessor. Individuals' exposures to chemicals are known to vary over time (longitudinal variation) and to vary from individual-to-individual (interindividual variation). Non-chemical stressors can be expected to have similar levels of longitudinal and interindividual variation. In addition, vast amounts of data on a community (demographics, economics, meteorology, geography, etc.) are often available but the value of the data and how it can be best used to predict the occurrence and intensity of stressors is unclear. To address these challenges, we propose to extend a system developed for categorizing data on the relationships between individual and community characteristics and chemical stressors (LifeLine, 2005) to non-chemical stressors. This system, 1) organizes individuals' and the community's characteristics into six categories, fixed, progressive, episodic, cyclic, and ephemeral, and 2) defines how each of the six types of variation influences other types and ultimately the patterns of individual's exposure. Once organized, the factors can be used to assist in the analysis of temporal, geographic, and demographic patterns of stressors and adverse effects in a community. More importantly, this system can be used in the development of person-oriented models (POM) of stressors (Price and Chaisson, 2005). Such models can be used to integrate data from diverse sources in a transparent and internally consistent manner to clarify associations, make predictions of causation, and develop strategies for mitigation.

W 1795 ISSUES WITH QUANTIFICATION OF THE JOINT IMPACT OF CHEMICAL AND NON-CHEMICAL STRESSORS FOR ENVIRONMENTAL HEALTH RISK ASSESSMENT.

R. C. Hertzberg. *Biomathematics Consulting, Atlanta, GA.* Sponsor: C. Rider.

The joint impact of exposure to chemicals and nonchemical stressors is complicated by both a lack of consistent exposure metrics and lack of quantitative exposure-response relationships. Many nonchemical factors are known to impact health, although often with rough descriptions. Heat impairs judgment, socioeconomic status affects psychological stress, and vibration alters cardiovascular function. In addition, some evidence of interactions exists, including noise and solvents jointly affecting hearing, chemical exposure affecting susceptibility to disease, and anxiety enhancing chemical toxicity. Potential exists for adapting approaches for chemical mixtures, including weight-of-evidence categories, hazard ranking formulas, and the Superfund hazard index. Ways of expressing known or suspected nonchemical exposures in quantitative terms will be explored, and some commonly used joint toxicity approaches and formulas will be discussed in terms of implications for toxicologic interaction. Examples will represent environmental and occupational scenarios.

W 1796 COMPARATIVE DIETARY RISK ASSESSMENT: AN ALGORITHM WITH INCORPORATION OF CULTURAL BENEFITS OR STRESSORS.

M. L. Dourson. *Toxicology Excellence for Risk Assessment (TERA), Cincinnati, OH.*

Analyses of fish throughout the United States confirm the presence of chemical contaminants. In many cases the concentrations of these contaminants have been high enough to warrant the posting of fish consumption advisories by state and local authorities. In virtually all cases, these advisories are based solely on the potential adverse effects posed by the contaminants in fish. Yet medical practice and recent publications suggest that the health benefits of eating—-even contaminated—-fish may outweigh the potential risks caused the by the presence of these contaminants. Furthermore, cultural and personal perceptions affect the choice of fish in our diet. A framework for comparing the risks from chemical contaminants in fish, the health benefits from fish consumption, and the cultural and personal perceptions in fish consumption choices is proposed. Data limitations and assumptions used to develop the framework are highlighted.

W 1797 EXTENDING PULMONARY TOXICITY FINDINGS FOR NANOMATERIALS.

D. Warheit[2] and P. Sayre[1]. [1]*U.S. EPA, Washington, DC and* [2]*DuPont, Providence, RI.*

As the diversity of manufactured nanomaterials continues to increase, associated exposures may pose concerns for human health. Subchronic inhalation studies are often needed to evaluate the possible effects related to longer-term inhalation exposures. However, since all nanomaterials cannot be tested in this manner, additional methods need to be developed for use by industry screening at the research and development phase, and/or for regulatory review as part of a commercial submission. One such method is to consider extending the pulmonary toxicity findings from one material within a class of nanomaterial types to other materials within that same class. This may not be possible for the majority of eclectic particle-types within most nanoscale-related categories. However, there exists well-developed pulmonary toxicity testing data for two classes of materials—those for nano titania and multi-walled carbon nanotubes (MWCNTs). The hazard data were generated for these two classes by utilizing standardized regulatory guideline methodologies, which make such predictions for closely-related materials approachable. Additional methodologies which may facilitate broader-based conclusions or assist in screening include use of other in vivo-based testing approaches such as those involving shorter-term inhalation, or aspiration/intratracheal instillation protocols. We will highlight different perspectives, consistent with our panel of experts diverse backgrounds which are needed to address this complex need. In addition, we will offer the latest findings on the pulmonary toxicity of nano titania and MWCNTs. In addition, viewpoints will be offered on how generalizable the findings are to other types of titania and MWCNTs, given differing physicochemical properties, methods of particle generation and delivery, and current perspectives on modes of actions. Insights will be presented on how such data can be utilized in applications such as pre-screening, and establishment of protective levels for workers. We believe that the dialogue between the panelists and the audience will facilitate the identification of areas of agreement, and key information needs, in order to further develop these approaches.

W 1798 TESTING OF MWCNT FOR POSSIBLE HEALTH HAZARDS AND RELEASE FROM COMPOSITE MATERIALS.

R. Landsiedel[1], W. Wohlleben[1, 2], L. Ma-Hock[1], S. Treumann[1], K. Wiench[3] and B. van Ravenzwaay[1]. [1]*Department of Experimental Toxicology and Ecology, BASF SE, Ludwigshafen am Rhein, Germany,* [2]*Polymer Physics Department, BASF SE, Ludwigshafen am Rhein, Germany and* [3]*Product Safety Department, BASF SE, Ludwigshafen am Rhein, Germany.*

MWCNT are of great commercial interest, as they combine high electrical conductivity, good mechanical strength and excellent thermal conductivity. A sustainable development of these materials requires a risk assessment of production. The toxicity of four different MWCNTs was examined by 5- inhalation studies and one MWCNT was subsequently examined in a 90-day inhalation studies in rats according to OECD test guideline no. 413. Test concentrations were 0 (control), 0.1, 0.5, or 2.5 mg/m^3. The exposure to MWCNT resulted in no systemic toxicity, but a concentration-dependent increase of lung weights and granulomas with histiocytic, neutrophilic inflammation and intra-alveolar lipoproteinosis in the lung and lung-associated lymph nodes at 0.5 and 2.5 mg/m^3. At 0.1 mg/m^3, there was still minimal granulomatous inflammation. Obviously, the exposure to the MWCNT could occur during production and compounding and the toxicity of the MWCNT demands strict industrial hygiene measures. Consumers and the general population are unlikely to be exposed to MWCNTs, unless they are bound in nanocomposites or in other ways incorporated into a product. The concern for nanocomposites is the release of MWCNT. This may occur due to weathering or by mechanical shear forces (drilling, sawing, sanding). We examined MWCNT release from thermoplastic and cementitious nanocomposites and found that the abraded materials from the nanocomposites were micron-sized hybrids, not naked MWCNTs. The amount of test substance obtained from the degradation was too small for inhalation studies. Therefore the sanding powder was instilled into rat lungs and its toxicity was compared to matrix without MWCNT and to the naked MWCNT: The naked MWCNT was significantly more toxic than the abraded material; but there was no difference in toxicity between the hybrid material from abrasion of composites with MWCNT and the abraded matrix without MWCNT.

W 1799 EXPERIMENTAL EVIDENCE FROM A 90-DAY MWCNT INHALATION STUDY IN RATS: ANALYSIS OF COMMON DENOMINATORS OF MWCNT WITH SUBMICRONSIZED PARTICLES.

J. Pauluhn. *Toxicology, Bayer HealthCare, Wuppertal, Germany.*

Multi-walled Carbon Nanotubes (MWCNT, Baytubes®) were examined in a subchronic 13-week inhalation study. The focus of study was to better understand the cause of toxicological properties of these types of aggregated structures and whether the density of agglomerates plays the key role in triggering the cascade of events leading to sustained pulmonary inflammation. There is compelling evidence from repeated inhalation exposure studies on rats suggesting that the particle displacement volume is the unifying denominator for pulmonary toxicity. Procedures were developed to analyze and model the pulmonary toxicokinetics and associated inflammatory potency of six different types of the following poorly soluble nano-/submicron dusts: ultrafine and pigmentary TiO2, synthetic iron oxide (Fe3O4, magnetite), two aluminum oxyhydroxides (AlOOH, Boehmite) with primary isometric particles of either 10 or 40 nm, and multiwalled carbon nanotubes (MWCNT, Baytubes®). The specific agglomerate densities of these materials ranged from 0.1 g/cm3 (MWCNT) to 5 g/cm3 (Fe3O4). Along with all PM, due to their long retention half-times and associated biopersistence in the lung, even short-term inhalation studies may require post¬exposure periods of at least 3 months to reveal PM-specific dispositional and toxicological characteristics. This analysis provides strong evidence that pulmonary toxicity (sustained inflammation) corresponds best with the volume-based cumulative lung exposure dose exceeding the overload threshold. This analysis supports the conclusion that repeated inhalation studies on rats should utilize an experimental window resulting in cumulative volume concentrations of respirable PM from 1 to maximal 10 µl/m3. This data support a volume-based generic no-adverse-effect mass concentration of respirable PM at 0.5 µl x agglomerate density/m3, independent whether nano- or submicronsized.

W 1800 FACTORS AFFECTING THE PULMONARY RESPONSE TO CARBON NANOTUBES.

V. Castranova. *Pathology & Physiology Branch, CDC NIOSH, Morgantown, WV.*

Published studies report that pulmonary exposure to single-walled carbon nanotubes (SWCNT) or multi-walled carbon nanotubes (MWCNT) causes transient inflammation, granulomatous legions, and persistent fibrosis. Due to the fibrous shape of carbon nanotubes (CNT), questions concerning asbestos-like induction of lung cancer and mesothelioma have also been raised. Therefore, hazard assessment for CNT is essential. There are at least three distinct synthesis processes for CNT. In addition, CNT can exist in the raw (catalytic metals present) or purified (metals removed) forms. Physical dimensions (both width and length) can vary among SWCNT and MWCNT. Furthermore, the degree of agglomeration of CNT can result in a wide range of structure sizes. Lastly, CNT can be functionalized in multiple ways, resulting in altered surface properties. Thus, hundreds of types of CNT are possible. Since it will not be feasible to evaluate the toxic potential of each CNT type, it is critical to determine the relationships between physicochemical characteristics of CNT and bioactivity. Development of such relationships requires the collaboration between material scientists and toxicologists. Such a matrix of physicochemical properties vs. bioactivity is critical for risk analysis. Existing data concerning the effects of metal contamination, dimension, and agglomeration state on the pulmonary reactions to CNT exposure will be presented. Another issue for risk assessment is the relevance of pulmonary responses after a bolus exposure to inhalation of CNT. In this presentation, pulmonary responses to bolus exposure (pharyngeal aspiration) will be compared to those after short term inhalation of SWCNT and MWCNT.

W 1801 TITANIA-CRYSTALLINITY AND SURFACE REACTIVITY EFFECTS ON TOXICITY—NOT ALL NANO TITANIUM DIOXIDE PARTICLES HAVE THE SAME HAZARD POTENTIAL.

D. B. Warheit. *DuPont Haskell Global Centers, Newark, DE.*

The lung toxicities of several different well-characterized nanoscale and fine TiO2 particle types were assessed in rats. The experimental design included dose response and time course characteristics, and benchmark control particulates. Groups of rats were intratracheally instilled with doses of 1 or 5 mg/kg of two different nano rutile TiO2 particle-types; a rutile fine-TiO2 particle; an anatase/rutile nano-TiO2 particulate; or alpha-quartz particles. Phosphate-buffered saline (PBS) solution instilled rats served as vehicle controls. Following exposures, the lungs of PBS and particle-exposed rats were evaluated by using bronchoalveolar lavage (BAL) fluid inflammatory markers, cell proliferation indices, and lung histopathology at post-exposure time points of 24 hrs, 1 week, 1 month, and 3 months. Rigorous physicochemical characterization of the TiO2 particle-types demonstrated significant differences in crystal structure, surface area indices, pH effects, and surface reactivity measurements- using the Vitamin C yellowing assay. The range of lung inflammation/cytotoxicity/cell proliferation and histopathological responses was alpha-quartz > anatase/rutile nano TiO2 > rutile fine TiO2 = rutile nano TiO2- 1 =rutile nano TiO2- 2. Exposures to quartz and to a lesser degree, anatase/rutile TiO2 particles produced pulmonary inflammation, cytotoxicity and adverse lung tissue effects. These results demonstrated that exposures to TiO2 nanoparticles can produce differential pulmonary inflammatory and histopathological effects, based upon their particle composition, surface reactivity, and crystal structure rather than particle size characteristics. Moreover, the lung toxicity of anatase/rutile TiO2 nanoparticles clearly is not representative of all TiO2 nanoparticle particle types. Therefore, the rigorous characterization of the nanoparticle test material is a necessary prerequisite for the interpretation of hazard studies. Finally, development of OELs for nano TiO2 particles requires consideration of physicochemical characteristics. Not all TiO2 particles are alike!

W 1802 CHARACTERIZING MWCNTS AND TITANIA NANOSTRUCTURES: DIFFERENT MATERIALS REQUIRE DIFFERENT TECHNIQUES.

C. Sayes. *Texas A&M University, College Station, TX.*

Manufactured nanomaterials are beginning to make their way into a variety of industrial processes and consumer goods. In parallel to the development of nanomaterials in the workplace, the need for regulatory guidelines is critical to success of these novel materials and their uses. One important factor within the regulatory discussion is the need for accurate and relevant nanomaterial characterization of the physicochemical properties for toxicological evaluations. Some of the most common properties of nanomaterials in aqueous suspensions are aggregation/agglomeration, leeching of metal ions, and the production of reactive species. With some nanomaterials, when designed appropriately, they can generate reactive oxygen species quite efficiently. In many studies published in the literature, it has been found that differences in the physicochemical properties of nanomaterials with a "class" determine the biological activity, including cytotoxicity and inflammation. While most data follows a dose-dependent and time-dependent trend, other materials do not. Phase composition, chirality, and surface coating of the nanomatertial is related to cytotoxicity. Anatase nano-titania, for example, is more than 100 times more toxic than an equivalent sample of titania in the rutile phase. The observed

toxicity of nanomaterials relates strongly with the material's ability to generate reactive species, ex vivo. An example of a parameter that has an effect on nanotoxicity is the presence or absence of light; light can exploit the ability to produce a biological response. After analyzing the data as a whole, the literature suggests that users of certain nanomaterials (or certain classes of nanomaterials) may partially rely on ex vivo measures of agglomeration, structure, bioavailable metals, interactions with light, and reactive species production, as well as other features such as crystallite phase, chirality, surface chemistry, and particle charge as indicators for assessment of health and safety.

W 1803 CONSIDERATION FOR BROAD MULTI-WALL CARBON NANOTUBE AND TITANIA OCCUPATIONAL EXPOSURE LIMITS AND RELEVANT INSIGHTS FROM THE EUROPEAN PERSPECTIVE.

L. Tran. *Institute of Occupational Medicine, Edinburgh, Scotland, United Kingdom.* Sponsor: P. Sayre.

The calculation of the Occupational Exposure Limit (OEL) requires information on the physico-chemical characteristics, the toxicological profile and the potential exposure level of the nanoparticle. In this presentation, the rationale for the derivation of OELs will be explained using existing toxicology data for MWCNT and Titanium Dioxide. Specifically, we will review the body of in vivo and in vitro toxicology (bio-kinetics, target organ toxicity), metrology and also some workplace exposure data related to these two nanoparticles. In extending this approach to general classes of engineered nanoparticles, the physico-chemical characteristics of nanoparticles, the uncertainty and variability of the toxicology data, as described in the other presentations in this meeting, as well as the knowledge gaps (e.g. relevant dose-metrics) will be taken into account. The data source is from existing peer-reviewed literature and also from European Projects FP7 ENPRA and NANOMMUNE. The existing methods for the Derivation of No-Effect-Level or Minimal Effect Level (DNEL and DMEL) from the European REACH process will also be discussed. Finally, we will address the applicability of our approach to the derivation of OELs for classes of engineered nanoparticles.

W 1804 UNDERSTANDING THE IMPLICATIONS OF PRECLINICAL SEIZURES FOR CLINICAL DRUG DEVELOPMENT.

A. Wilke[1] and M. Kallman[2]. [1]*Nonclinical Safety Assessment, Eli Lilly and Co., Indianapolis, IN and* [2]*Neuroscience and Investigative Toxicology, Covance, Inc., Greenfield, IN.*

Convulsions observed in animals during the drug development process require a thorough risk assessment to determine the implications for human dosing. The origin of an outwardly visible convulsion is generally considered to be a generalized seizure (i.e., an abnormal/sychronized electrical discharge in the brain). While seizures are sometimes due to the intended, or on-target pharmacology of the molecule, seizures are likely more often due to an unintended, or off-target central nervous system (CNS) effect. Seizures in humans are problematic given the potential safety impact of loss of motor control and/or consciousness. While the intent is to avoid seizures in humans, there is no published consensus on how to accomplish this (e.g., what exposure multiple is appropriate to ensure the safety of clinical subjects; how valuable are premonitory signs or EEG data for predicting seizures). Therefore we will review some of the available animal models and tools, and to build consensus on appropriate approaches to seizure risk assessment during drug development.

W 1805 AN OVERVIEW OF SEIZURES AND THE IMPACT OF DRUG-INDUCED SEIZURES ON DRUG DEVELOPMENT.

M. J. Kallman. *Covance Inc., Greenfield, IN.*

This presentation will provide an initial overview of the biology of seizures and establish key concepts regarding seizures and convulsions. The process for behavioral coding of overt convulsions and the traditional Racine scale used to capture the details of the hierarchical details of behavioral display will be discussed. Since many successfully marketed drugs do have liability for convulsion, a process for understanding the implications of preclinical convulsion coupled with identification of margin, progression of premonitory events, and knowledge of the clinical population can impact the progress of compounds in development. Examples of data that can support safe clinical progression of compounds will be discussed and recommendations about which seizure models are most helpful at different points in drug development will be shared.

W 1806 TRANSLATION OF EEG DATA FROM ANIMALS TO MAN.

J. Arezzo. *Department Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY.* Sponsor: A. Wilke.

Electroencephalogram (EEG) data can be used both to identify abnormal brain electrical activity in animals or human subjects prior to study and to evaluate the same individuals during a study. The general methodology and utility of these approaches will be presented. In particular, the speaker will discuss when screening EEGs and detailed EEG evaluations are most valuable. In addition, this speaker will emphasize the translation of animal EEG data to clinical trials to ensure safety of human subjects. The sophistication of control mechanisms across species will be discussed with respect to clinical risk assessment.

W 1807 APPROACHES TO SEIZURES IN DRUG DEVELOPMENT: AN INDUSTRY PERSPECTIVE.

A. Wilke. *Nonclinical Safety Assessment, Eli Lilly and Co., Indianapolis, IN.*

The speaker will present case studies that exemplify several aspects of managing continued development when drug-induced seizures have occurred in animals. In particular, the case studies illustrate the approach of maintaining an appropriate exposure multiple, the collection and use of relevant clinical signs and of EEG data, and the impact of the patient population on the degree of risk mitigation needed.

W 1808 CONSIDERATIONS WHEN MANAGING SEIZURES IN DRUG DEVELOPMENT.

A. Avila. *Division of Psychiatry Products, U.S. FDA, Silver Spring, MD.* Sponsor: A. Wilke.

This presentation will address safety assessments to consider when seizures develop in nonclinical studies and their relationship to regulatory decision making and clinical risk. Emphasis will be on additional safety assessments to include in nonclinical studies as well as parameters to consider when assessing clinical risk including safety margins, drug/metabolite exposure levels, pharmacokinetics, premonitory signs, use of EEG data in animals and/or humans, drug indication and patient population. All of these topics will be discussed from a regulatory standpoint.

PL 1809 A NEW COMPUTATIONAL TOOL TO DETERMINE TISSUE:BLOOD PARTITION COEFFICIENTS FOR A VARIETY OF CHEMICALS.

E. E. Carpenter, A. N. Mayeno and B. Reisfeld. *Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO.*

Computer-based (*in silico*) methods are increasingly used to inform chemical risk and safety assessments. One such method is physiologically-based pharmacokinetic (PBPK) modeling, which uses biologically-based mathematical representations of components in the body to quantify the absorption, distribution, metabolism, and excretion (ADME) of the foreign chemicals (xenobiotics) of interest. The concentration of the xenobiotic in each body compartment is related to the overall concentration in the blood by a tissue:blood partition coefficient (K_p), a parameter dependent on both the organism's physiology and the nature of the chemical. Though experimental methods can be used to determine K_ps, *in silico* methods have the significant advantages of convenience, flexibility, and speed. Such an approach was developed by Rodgers *et al.* (J. Pharm. Sci. 2005, 94:1259–1276; J. Pharm. Sci. 2006, 95:1238-1257) who determined K_ps by conceptually dividing relevant tissues into components that vary in chemical binding and partitioning, and using chemical and thermodynamic relationships to formulate equations for the amount of xenobiotic in each component. The derivation and direct application of these equations, however, is tedious and error prone, especially for chemicals with multiple pK_as. Here, we present a novel *in silico* approach for the calculation of tissue:blood partition coefficients that automates and extends the methodology of Rodgers et al. and overcomes many of its limitations and difficulties. This computer-based framework takes, as inputs, basic physiological and chemical property data and uses symbolic mathematics to derive the appropriate governing equations and compute the K_p values specific to the xenobiotic of interest for several tissue types. The K_p results

calculated using this new tool are generally consistent with those calculated by Rodgers *et al.*, while providing a convenient, approachable and high-throughput tool to estimate these measures for a large variety of xenobiotics.

PL 1810 A COMPUTATIONAL FRAMEWORK FOR CHARACTERIZING BIOMARKERS OF ORGANOPHOSPHORUS INSECTICIDE MIXTURE EXPOSURE.

J. H. Ivy[1, 2], J. M. Wright[2], J. L. Rogers[1, 2], A. N. Mayeno[1, 2], M. A. Lyons[1, 2] and B. Reisfeld[1, 2]. [1]*Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO and* [2]*Systems and Computational Biology Research Group, Colorado State University, Fort Collins, CO.*

The disposition and toxicological effects of organophosphorus insecticides (OPs) following human exposure are governed by complex absorption, distribution, metabolism, and elimination (ADME) and pharmacodynamic (PD) processes. Among the signatures of these processes are a variety of metabolites, some of which are common to many OPs and some of which are unique to a given OP. Characterizing these biomarkers of exposure and effect, and their quantitative relationship to the an individual's ADME and PD, warrants the development and use of quantitative and computational modeling tools. Here we describe the development and usage of such a modeling tool that can facilitate the analysis of tissue dosimetry based on given dosing scenarios and reconstruct dose from specific biomarker data. This software framework, DoseSim:OP, is centered around a sophisticated differential equation solution and statistical simulation engine, allowing analyses that include Bayesian inference and Monte Carlo stochastic simulations. The modular architecture behind DoseSim:OP allows different models (ADME+PD) to be linked to the simulation engine, allowing great flexibility in the systems to be analyzed. The first of the models to be implemented is a physiologically-based pharmacokinetic model for a mixture of chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate] and diazinon [*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate] based upon the work of Timchalk et al. (2008). We have further refined this model using data from our own studies, coupled with optimizations and global sensitivity analyses. Predictions from DoseSim:OP for various exposure and dose reconstruction scenarios have shown good agreement with those from the literature and to our own experiments, and demonstrate the flexibility and utility of this methodology and framework. This project is supported by EPA STAR Grant #RE83345101.

PL 1811 QSAR ANALYSIS OF THE ATSDR DATABASE OF CHEMICAL HEALTH GUIDANCE VALUES.

E. Demchuk[1], Y. Tie[1], T. Miller[2] and R. M. Garrett[2]. [1]*Division of Toxicology & Environmental Medicine, ATSDR/CDC, Atlanta, GA and* [2]*Department of Defense, Washington, DC.* Sponsor: B. Fowler.

The ATSDR SEQUOIA (formerly HazDat) database contains information concerning thousands of chemicals collected at hazardous waste sites across the nation. However, less than 10% of them have been extensively reviewed to provide public health guidance. A goal of this project is to develop QSAR (Quantitative Structure Activity Relationship) models using these well-studied chemicals and apply the models to estimate provisional health guidance values (pHGVs) for other toxic chemicals that have not yet been reviewed by ATSDR. The QSAR analysis was conducted on a set of 100 compounds with rat oral chronic LOAEL (lowest-observed-adverse-effect-level) values; and a QSAR model with R2 and Q2 of 0.92 and 0.82, respectively, was developed. The model was applied to assess the toxicity of n-alkanes found in the 2010 BP Gulf oil spill, because limited toxicological information is available for long-chain alkanes and their mixtures. The LOAEL estimates obtained using our model were in fair agreement with those obtained using the TOPKAT package, and they were in an excellent agreement with information on ADMET properties of n-alkanes found in the ATSDR toxicological profile for Total Petroleum Hydrocarbons. Further validation of the model and expansion of its chemical space domain are under way. In the future, a robust model for pHGVs may complement the risk assessment of pure chemicals and multi-component exposures carried out using conventional methods.

PL 1812 SUPPORTING SAFETY ASSESSMENT OF DRUG IMPURITIES THROUGH EXAMINATION OF AN AMES ASSAY QSAR MODEL.

G. Myatt[1], K. P. Cross[1] and L. G. Valerio[2]. [1]*Leadscope Inc., Columbus, OH and* [2]*CDER/OPS, U.S. FDA, Silver Spring, MD.* Sponsor: N. Sadrieh.

Drugs under development may contain multiple impurities with structural alerts portending to toxicity, and thus to public health. According to the FDA draft guidance to industry on genotoxic and carcinogenic impurities in drug products and substances, impurities can be evaluated for genotoxicity based on structure-activity relationship (SAR) analysis. These molecules if predicted positive may need to have their levels controlled or the impurity synthesized and qualified in the Ames assay. Given the importance of adequately qualifying genotoxic impurities, this study evaluated a quantitative SAR (QSAR) analysis model developed that predicts Ames assay outcomes based on a training dataset of over 3000 molecules. A drug-like external validation set of over 3000 molecules was used to characterize the QSAR model performance by structural class, therapeutic category, and domain-of-applicability. Preliminary predictive performance statistics for the external test set (79% sensitivity, 76% specificity, 77% accuracy) were comparable to the model cross-validation statistics (77% sensitivity, 88% specificity, 83% accuracy). Hundreds of drug impurity molecules (proprietary and public) were examined at the FDA to assess the suitability of the chemical space of the Ames model for predicting drug molecules. The predominant molecular features contributing to Ames positive predictions of the drug impurities were elucidated through hierarchical classification of discriminating model features. This analysis has led to an improvement for predicting the mutagenicity of drug impurities in the Salmonella assay and by extending our understanding of known and new structural alerts in the Ames assay. The QSAR model is intended to help support decision-making during safety evaluations of the genotoxic potential of drug impurities.

PL 1813 ESTIMATING TOXICITY-RELATED BIOLOGICAL PATHWAY ALTERING DOSES FOR HIGH-THROUGHPUT CHEMICAL RISK ASSESSMENTS.

R. Judson, D. J. Dix, K. J. Robert, R. Setzer, E. A. Cohen Hubal and M. T. Martin. *U.S. EPA, Research Triangle Park, NC.*

The doses at which toxicity-related biological pathways are altered, measured using in vitro assays, are analogs to regulatory values such as Reference Doses (RfD) derived from in vivo toxicity tests. These biological pathway altering doses (BPADs) could be used in high throughput risk assessments of 1000s of data-poor environmental chemicals. Estimating a BPAD requires definition of biological pathways that can lead to toxicity, and development of in vitro assays for these pathways that can rapidly, efficiently and quantitatively test many chemicals. The ToxCast and Tox21 programs are providing data from 100s of in vitro assays, on 1000s of environmental chemicals. The first step is to estimate minimum chemical concentrations required to significantly alter toxicity-related biological pathways in vitro, i.e., the Biological Pathway Altering Concentration (BPAC). Pharmacokinetic modeling is then used to estimate the in vivo oral dose required to achieve the BPAC in target species and tissues (e.g., human serum concentration). Uncertainties are incorporated in both the BPAC and the pharmacokinetic parameters, and these are combined to yield a probability distribution for the dose required to alter pathway function. The BPAD is defined as the lower, protective end of this pathway-altering confidence interval. We illustrate with two examples. In the first, BPADs corresponding to the lowest AC50 for CAR/PXR-related assays were compared to the No-Effect Level (NEL)/100 for chronic rodent liver pathology of 13 conazole fungicides. In most cases, CAR/PXR BPADs were lower than the NEL/100, providing a reasonable starting point for setting exposure limits if these were data poor chemicals. In the second case, the lowest BPADs for any pathway were compared with regulatory RfDs for 35 chemicals and, again, BPADs were mostly lower than RfDs. BPADs were significantly higher than RfDs (less protective) in a few cases, but most of these were cholinesterase inhibitors requiring bioactivation for toxicity. This abstract does not necessarily reflect U.S. EPA policy.

PL 1814 EVALUATION OF *IN SILICO* TOOLS FOR THE PREDICTION OF MUTAGENICITY.

A. Hillebrecht, W. Muster, A. Brigo, M. Kansy, T. Weiser and T. Singer. *Pharmacology Research Nonclinical Safety, F. Hoffmann-La Roche Ltd., Basel, Switzerland.*

Four software packages (DEREK for Windows, Leadscope Model Applier, MCASE MC4PC and Toxtree) were systematically evaluated for their ability to predict the outcome of the Ames assay. A large, high quality data set of 9861 compounds, comprising public as well as Roche proprietary data, was used to test the systems. Acceptable performances were observed with the publicly available data sets, whereas a significant deterioration was obtained when the assessment was performed on the proprietary data. In particular, the sensitivity values dropped below 45 %. The relatively low amount of Ames positive compounds included in pharmaceutical industry data sets and the different coverage of the chemical space are the most likely explanations for the observed discrepancy between test sets. As a general trend, expert systems (DEREK, Toxtree) tend to exhibit higher sensitivities, but lower specificities compared to QSAR-based systems (MC4PC, Leadscope Model Applier).

PL 1815 MEASURING COMPOUND SELECTIVITY AND ITS LINK TO *IN VIVO* TOXICITY STUDY OUTCOME.

X. Wang and N. Greene. *Compound Safety Prediction, Pfizer, Groton, CT.*

In order to reduce drug attrition, it's important to assess the safety liabilities of early leads. A common practice to assess compound toxicity is using pharmacologic profiling panels. Several methods for measuring compound selectivity were developed over the years, from simple hit rate (defined as the fraction of assays with inhibition values greater than certain threshold for each compound) to more sophisticated methods, such as Gini Coefficient or Thermodynamics-Based Partition Index. Gini Coefficient measures statistical dispersion and is scale-free with no units. It is originally used to measure inequality of income or wealth, and had been successfully applied to measure Kinase inhibitors selectivity. The partition indexed can be defined as a competition assay in which the compound is placed in a test tube, and then compete for binding to a pool of targets. At thermodynamic equilibrium, the fraction of bound compound for a particular target is the "partition" index.
In this study, we compared these three methods: hit rate, Gini Coefficient, and partition index, based on a panel of 180 Bioprint (CEREP) inhibition assays. First, the impact of different assay selection on selectivity score was tested by randomized studies, and a panel of 15 assays was selected that best represent the overall assay selectivity profiles. Second, ~200 Pfizer compounds had gone through in vivo toxicity studies, and can be divided into two groups: clean@10uM and findings@10uM. The capability of each method to separate two groups of compounds based on those 15 assays was measured. The results show that each methods capture different nuances of the data. Hit rate is most intuitive; Gini Coefficient needs to be modified to deal with small panel of assay; while partition index is the most predictive. It's possible that the three methods could be combined to generate the optimal selectivity index.

PL 1816 IDENTIFICATION AND CLASSIFICATION OF GENOTOXIC AND NON-GENOTOXIC SCAFFOLDS IN RODENT CARCINOGENICITY QSAR MODELS.

K. P. Cross[1] and E. J. Matthews[2]. [1]*Leadscope, Inc., Columbus, OH and* [2]*U.S. FDA/CFSAN/OFAS, College Park, MD.* Sponsor: R. Tice.

Quantitative structure-activity relationship (QSAR) models have been developed for predicting mutagenicity in Salmonella typhimurium and carcinogenicity in rodents using Leadscope software. These models identify structure-activity relationships (SARs) between molecular features and chemical toxicity, and the SARs can support plausible mechanism of action (MOA) hypotheses responsible for the toxicity. The molecular features can be represented as molecular substructures that discriminate for, or against, toxicity, and the features may be extracted as scaffolds. The predictive performance of these scaffolds may be assessed for accuracy and precision independently of the model they were derived from. This study revealed that a relatively small number of primary scaffolds, alerts, and MOAs were associated with mutagenicity; conversely, a relatively large number of scaffolds were associated with carcinogenicity. We also extracted and compared the predominant scaffolds contributing to carcinogenicity and mutagenicity through hierarchical classification of discriminating model features. Preliminary analysis of the salmonella QSAR model indicated that 58% of salmonella model scaffolds matched compounds predominantly positive for rodent carcinogenicity, while only 2% of the scaffolds matched compounds predominantly negative for rodent carcinogenicity (40% remained unclassified). Preliminary analysis of the rodent carcinogenicity QSAR model indicated that 43% of carcinogenicity model scaffolds matched compounds predominantly positive for salmonella (and classified as genotoxic), while 30% of the scaffolds matched compounds predominantly negative for salmonella (and classified as non-genotoxic). The remaining 27% of the scaffolds were classified as only partially responsible for genotoxicity. The analysis further classified scaffolds for each of the rodent carcinogenicity models (mouse, rat, rodent, and sex) and identified genotoxic and non-genotoxic scaffolds common across several different models.

PS 1817 EFFECTS OF ORAL DOSAGE REGIMEN ON FIRST-PASS ELIMINATION AND TOXICOKINETICS (TK) OF 1, 1, 1-TRICHLOROETHANE (TRI) AND TRICHLOROETHYLENE (TCE).

V. Srivatsan, S. Muralidhara, J. V. Bruckner and C. A. White. *Pharmacology and Biomedical Sciences, University of Georgia, Athens, GA.*

The objective of this investigation was to compare the TK of TRI and TCE under conditions representative of drinking water exposure, and to contrast these TK profiles with those when the halocarbons are given by gavage. Fasted male Sprague-Dawley rats were given 6 – 48 mg TRI/kg or 8 – 50 mg TCE/kg, as an aqueous emulsion ia, iv or by constant gastric infusion (gi) over 2 h. Serial micro-blood samples were taken and analyzed by GC, while other rats were sacrificed serially for blood and tissue collection. Bioavailability of TRI exceeded 95% and was independent of dose and dosage regimen. This was not the case for TCE. First-pass elimination of the 8 mg TCE/kg gi dose was virtually complete. Rapid absorption of TRI ad TCE into blood and tissues was followed by relatively rapid elimination of TCE. In vivo TRI blood:tissue partition coefficients were consistently higher than corresponding values for TCE. First-pass pulmonary elimination of TRI was independent of dose and dosage regimen and exceeded that of TCE, while the converse was true for hepatic first-pass elimination. These TK data may prove useful in physiological modeling and in assessment of health risks of these common drinking water contaminants. Supported by U.S. DOE DE-FC09-02CH11109.

PS 1818 *IN VITRO* METABOLISM OF BDE-99 IN HUMAN LIVER MICROSOMES.

C. Erratico and S. Bandiera. *Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, BC, Canada.*

Polybrominated diphenyl ethers (PBDEs) are persistent, bioaccumulative, and toxic environmental pollutants frequently detected in human samples. Limited information is available for PBDE metabolism in humans. In this study, metabolism of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), the major component of a widely used commercial PBDE mixture (Penta-BDE), was investigated in vitro to determine the hydroxy metabolites formed, rates of metabolite formation, and the cytochrome P450 (CYP) enzymes involved. BDE-99 was incubated with human liver microsomal preparations. Hydroxy metabolites were liquid-to-liquid extracted and quantified using liquid chromatography/mass spectrometry. Incubation conditions were optimized for substrate, NADPH, protein concentration, and incubation time. BDE-99 was metabolized to two major (5′-OH-BDE-99 and 2,4,5-tribromophenol), one intermediate (4′-OH-BDE-101), and two minor (4-OH-BDE-90 and 6′-OH-BDE-99) oxidative products by human liver microsomes. Rates of formation of major and minor metabolites ranged between 18 and 20 and between 0.6 and 0.9 pmol/min/mg protein, respectively. No other hydroxy or di-hydroxy metabolite of BDE-99 was detected under the experimental conditions used. Structures of the major and minor BDE-99 metabolites produced by human liver microsomes are different from structures of the major and minor BDE-99 metabolites identified in a previous study using rat liver microsomes. Moreover, BDE-99 undergoes more extensive metabolism in human than in rat liver microsomes (50 vs 3.5 pmols/min/mg protein). Formation of BDE-99 hydroxy metabolites is of toxicological concern as in vitro studies have recently shown that hydroxy PBDEs affect steroidogenesis and are agonists of human estrogen and transthyretin receptors. Experiments are in progress to identify the CYP enzymes responsible for the formation of BDE-99 metabolites using a panel of human recombinant CYP enzymes. The same experimental design will be used to characterize the metabolism of the second major component of Penta-BDE mixture, BDE-47.

PS 1819 BIOTRANSFORMATION OF ETHANOL TO ETHYL GLUCURONIDE IN A RAT MODEL AFTER A SINGLE ORAL DOSAGE.

T. Haupt and K. Ferslew. *East Tennessee State University, Johnson City, TN.*

Ethyl glucuronide (EtG) is a minor ethanol (Et) metabolite that confirms the absorption and metabolism of Et after oral or dermal exposure. Human data suggest maximum blood EtG concentrations are reached between 3.5 - 5.5 hours post Et administration [1]. This study was undertaken to determine if the Sprague Dawley (SD) rat biotransforms Et to EtG after a single oral dose of Et. SD rats (male, n=6) were gavaged with a single Et dose (4g/kg) and urine was collected for 3 hours in metabolic cages, followed by euthanization and collection of heart blood. Blood and urine were analyzed for Et and EtG by gas chromatography and enzyme immunoassay. Blood and urine Et concentrations (BEt, UEt) were 195 ± 23 and 218 ± 19 mg/dL while blood and urine EtG concentrations (BEtG, UEtG) were 1363 ± 98 ng/mL and 2.1 ± 0.29 mg/mL (mean ±SE). Sixty-six, male, SD rats were gavaged Et (4 g/kg) and placed in metabolic cages to determine the extent and duration of Et to EtG biotransformation and urinary excretion. Blood and urine were collected up to 24 hours post administration for Et and EtG analysis. Maximum BEt, UEt, and UEtG were reached within 4 hours while maximum BEtG was reached 6 hours post administration. Maximum concentrations were: BEt, 213 ± 20 mg/dL; UEt, 308 ± 34 mg/dL; BEtG, 2683 ± 145 ng/mL; UEtG, 1.2 ± 0.06mg/mL (mean ±SE). Areas under the concentration-time curve were: BEt, 1632 hr*mg/dL; UEt, 2936 hr*mg/dL; BEtG, 11764 hr*ng/mL; UEtG, 8.5 hr*mg/mL. BEt and BEtG were reduced to below limits of detection (LOD) within 12 and 18 hours post Et administration. UEt's were below LOD at 18 hours, but UEtG was still detectable at 24 hours post administration. Our data proves that the SD rat biotransforms Et to EtG and excretes both in the urine and suggests it is similar to that of the human.

PS 1820 RAT LIVER MICROSOMES ENANTIOSELECTIVELY CATALYZE THE FORMATION OF HYDROXYLATED METABOLITES OF 2, 2', 3, 3', 6, 6'-HEXACHLOROBIPHENYL (PCB136).

I. Kania-Korwel[1], M. W. Duffel[2], S. Bandiera[3] and H. Lehmler[1]. [1]*Department of Occupational and Environmental Health, University of Iowa, Iowa City, IA,* [2]*Division of Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA and* [3]*Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada.*

Chiral PCB congeners such as PCB136 undergo enantiomeric enrichment in vivo. The biotransformation processes responsible for this enantiomeric enrichment are still poorly understood. A series of metabolism studies was performed to test the hypothesis that metabolism of PCB136 is enantioselective and isoform-dependent in liver microsomes from rats treated with phenobarbital (PB, CYP2B induced), dexamethasone (DEX, CYP3A induced) or corn oil (CO). Incubations of all three types of microsomes with racemic PCB136 (50 μM) resulted in the time-dependent formation of hydroxylated metabolites. Microsomes from PB-treated animals generated the highest amount of metabolites, followed by microsomes from DEX and CO- treated rats. The major metabolite in all preparations was 5-OH-PCB 136 and the rank order was 5-OH-PCB 136 > 4-OH-PCB136 > 4,5-diOH-PCB136. However, the relative contribution of 4-OH-PCB136 depended on the microsomal preparation and increased in microsomes from DEX and CO-treated rats. Carbon monoxide inhibited the formation of metabolites, which is consistent with the involvement of cytochrome P450 isoforms in the metabolism of PCB136. Incubation of microsomal preparations from both PB- and DEX-treated rats with (+)-, (-)-, and racemic PCB 136 revealed no clear differences in metabolite profiles. Although no enantiomeric enrichment of PCB136 was observed, the second eluting atropisomers of 4-OH-PCB136 and 5-OH-PCB136 were slightly enriched in incubations with racemic PCB136. Incubations with pure PCB 136 atropisomers demonstrated that the second eluting atropisomers of both metabolites are formed from (+)-PCB136. Overall, these studies demonstrate the enantioselective and isoform-dependent formation of hydroxylated PCB 136 metabolites by cytochrome P450 enzymes (Supported by NIH ES017425).

PS 1821 MYELOPEROXIDASE-MEDIATED BIOACTIVATION OF 5-HYDROXYTHIABENDAZOLE: A POSSIBLE MECHANISM BEHIND THE TOXICITIES ASSOCIATED WITH THIABENDAZOLE.

J. Jamieson, E. Smith, D. Dalvie, G. Stevens and G. Yanochko. *Pfizer, Inc., San Diego, CA.*

Thiabendazole (TBZ), an antihelminthic and antifungal agent, is associated with a host of adverse effects including nephrotoxicity, hepatotoxicity, and teratogenicity. Bioactivation of the primary metabolite of TBZ, 5-hydroxythiabendazole, has been proposed to yield a reactive intermediate. Here we show that this reactive intermediate can be generated by myeloperoxidase, a neutrophil-bourne peroxidase. Using a cell viability endpoint, we examined the toxicity of TBZ, 5OH-TBZ, and MPO-generated metabolites in cell-based models including primary rat proximal tubule epithelial cells, NRK-52E rat proximal tubule cells, and H9C2 rat myocardial cells. Timecourse experiments with MPO showed complete turnover of 5OH-TBZ within 15 minutes and a dramatic leftward shift in dose-response curves after 12 hours. After a 24 hour exposure in vitro, the LC50 of this reactive intermediate was 22 ± 1μM compared to 117 ± 20μM from 5OH-TBZ alone, a greater than 5-fold decrease. LC50 values were equal in all cell types used. This toxicity can be completely rescued via incubation with rutin, an inhibitor of MPO. These results suggest that MPO-mediated biotransformation of 5OH-TBZ yields a reactive intermediate which may play a role in TBZ-induced toxicity.

PS 1822 METABOLISM OF 2-HYDROXY-4-METHOXYBENZOPHENONE DEPENDS ON SPECIES AND SEX.

C. Wegerski[1], S. S. Auerbach[2], J. M. Sanders[2], M. Doyle-Eisele[1] and J. D. McDonald[1]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM and* [2]*NTP/NIEHS, Research Triangle Park, NC.*

Metabolism and disposition studies of 2-Hydroxy-4-methoxybenzophenone (HMB) are being conducted to aid in interpretation of toxicology results and interspecies and sex extrapolation. To assess species and sex differences within rodents, in vivo studies were conducted by oral dose administration of 14C labeled HMB in male and female Sprague-Dawley rats and B6C3F1 mice. Urine samples were analyzed for HMB metabolites. To confirm these results and extend to human metabolism, in vitro studies were conducted by incubating 14C HMB with male and female liver hepatocytes from Sprague-Dawley rats, B6C3F1 mice, and humans. Samples were analyzed by LC-RAD and/or LC-MS/MS. There were sex differences in the in vivo metabolism of HMB detected in urine between the male and female rat. The major metabolite in the female rat was not detected in the male rat. Similarly, the major metabolite in the male rat was a minor metabolite in the female rat. There were also species differences between the rat and mouse, with the major metabolite in the male mouse urine present only in trace quantities in the male rat urine. Both HMB and 2,4-dihydroxy-benzophenone, which were present in the male mouse and rat urine, were absent or in trace quantities in the female rat urine. Species and sex differences in HMB metabolism were also observed in isolated rat, mouse and human hepatocytes. Female rodent hepatocytes produced all HMB-derived metabolites present in males, as well as a few minor metabolites not evident in males. Male human hepatocytes produced 10 HMB-derived metabolites compared to 8 detected in female human hepatocytes. The metabolites detected in these studies were primarily glucuronide and sulfate conjugates, but some Phase I oxidative intermediate metabolites were also observed. The in vitro data indicate that the mouse is a closer metabolic match to humans than the rat. (Work conducted under NIEHS contract N01-ES-75562).

PS 1823 THE *IN VITRO* METABOLISM OF METHYL AND PROPYL PARABENS IN HUMAN AND RAT.

K. Choi, H. Joo, J. Campbell, M. Andersen and H. Clewell. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Parabens are antimicrobial preservatives widely used in cosmetic products and pharmaceuticals as well as in food and beverage processing. Methyl paraben (MP) and propyl paraben (PP) are the most predominantly used parabens. In mammals parabens are extensively hydrolyzed to p-hydroxy benzoic acid (pHBA) and further undergo phase II biotransformation via glucuronide, glycine and sulfate conjugation. However, their metabolism in humans has not been well characterized. We investigated the metabolism of MP and PP using subcellular fractions of various organs in human and rat. In human, pHBA was mainly produced in the liver, followed by the lung, skin, intestine and testis (for MP) or the intestine, kidney, lung, skin and testis (for PP). For MP in human, the velocities for pHBA production were 94.72±5.35 and 46.23±0.07 in liver microsomes and cytosol and 38.17±3.64 and 13.11±0.45 (nmol mg protein-1 min-1) in lung microsomes and cytosol, respectively. For PP, the velocities for the production of pHBA were 114.71±0.85 and 45.90±1.48 in liver microsomes and cytosol, 8.79±0.05 and 32.38±5.50 (nmol/mg protein/ min) in intestine microsomes and cytosol, respectively. In rat liver microsomes, the velocities for the production of pHBA with treatment of MP and PP were 106.14±2.55 and 104.15±4.88 and in rat liver cytosol 36.53±6.0 and 111.76±3.05 (nmol/mg protein/min), respectively. These metabolism data provide key parameters for PBPK models that support cross-species extrapolation of parabens exposures.

PS 1824 PHASE II BIOTRANSFORMATION OF DI(2-EHTYLHEXYL) PHTHALATE IN HUMAN AND RAT.

H. Joo[1], K. Choi[1], J. Grimes[1, 2], T. O'Connell[1, 2], R. Clewell[1], J. Campbell[1] and H. Clewell[1]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [2]*The UNC, Chapel Hill, NC.*

In this study we demonstrated that three metabolites of di(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-OH-hexyl) phthalate (5-OH MEHP) and mono(2-ethyl-5-oxo-hexyl) phthalate (5-oxo MEHP), were metabolized via conjugation with glucuronic acid in pooled human liver (HLM) and rat liver microsomes (RLM). Production of MEHP-, 5-OH MEHP- and 5-oxo MEHP glucuronide was measured by LC-ESI (-)/MS/MS. The metabolic rates in HLM and RLM were 37,460 ± 5,565 and 4,472 ± 206 for MEHP glucuronide, 102,229 ± 1,160 and 868 ± 3 for 5-OH MEHP glucuronide and 5,549 ± 350 and 920 ± 28 (AUC mg protein-1 min-1) for 5-oxo MEHP glucuronide, respectively. We also determined metabolic activities of 12 human uridine 5-diphosphoglucuronosyl transferase (UGT) isoforms (1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17) toward MEHP, 5-OH MEHP and 5-oxo MEHP. Among the 12 UGTs 1A3, 1A8, 1A10, 2B7 and 2B17 were major isoforms for MEHP glucuronide production, while 1A3, 1A7, 1A8, 1A9, 1A10 and 2B7 were mainly responsible for the production of 5-OH MEHP- and 5-oxo MEHP glucuronide. The percent total normalized rates (%TNR) were 84% for MEHP glucuronide by UGT2B7 and 80% and 85% for 5-OH MEHP- and 5-oxo MEHP glucuronide by UGT1A9, respectively. Additionally, we developed a method to biosynthesize milligram quantities of MEHP glucuronide for use as a reference standard. These metabolism data are being used to support PBPK model evaluation of cross-species dosimetry and human interindividual variability for DEHP exposures.

PS 1825 EFFECTS OF METABOLISM BY INTESTINAL MICROORGANISM ON PHARMACOKINETICS OF BAICALIN *IN VIVO.*

T. Jeong, M. Kang, G. Ko, S. Yoo, H. Ha, M. Kong and D. Lee. *Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea.*

Baicalin is an active ingredient of Scutellaria baicalensis Georgi. Scutellaria baicalensis is one of the most popular herbs used in Korea traditionally for treatment of inflammation, cardiovascular diseases, hypertension, and bacterial and viral infections. In this study, effects of intestinal microorganism on the pharmacokinetics of baicalin were determined in conventional and antibiotics-treated rats following p.o. administration with 100 mg/kg baicalin by using liquid chromatography/electrospray ionization mass spectrometry. The Tmax values of baicalin in conventional and antibiotics-treated rats were 4.00 and 3.60 hr, respectively. The AUC values were 51.76 and 26.42 μg×hr/ml. These results showed that the intestinal microorganism might have an important role in pharmacokinetics of baicalin. [Supported by a grant (09172KFDA996) from Korea Food & Drug Administration.]

PS 1826 IMMUNOTOXIC EFFECTS OF ARBUTIN *VIA* METABOLISM BY INTESTINAL BACTERIA *IN VITRO.*

M. Kang[1], H. Ha[1], H. Kim[2], G. Ko[1], S. Yoo[1], D. Lee[1], M. Kong[1], Y. Ahn[3], H. Jeong[2] and T. Jeong[1]. [1]*Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea,* [2]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea and* [3]*Yakult Co., Yongin, Republic of Korea.*

A possible role of metabolism by intestinal bacteria in arbutin-induced toxicity was investigated in spleen cell cultures from mice. Following an incubation of arbutin with intestinal bacteria for 24 h, its aglycone hydroquinone could be produced in the bacterial culture media and detected by an HPLC. When the toxicity was compared in the spleen cell cultures, hydroquinone was more toxic than the parent arbutin, indicating that metabolic activation might be required in arbutin-induced toxicity. After arbutin was incubated with intestinal bacteria for 24 h, ability of the incubate to suppress LPS and Con A mitogenicity was tested. As results, the incubate with intestinal bacteria was more toxic when compared to arbutin control. The present results indicate that the current system might be applied for assessing the possible role of metabolism by intestinal bacteria in chemical-induced toxicity in spleen cell cultures of mice. (Supported by a grant (09172KFDA996) from Korea Food & Drug Administration.)

PS 1827 ROLE OF METABOLISM BY INTESTINAL BACTERIA IN GENIPOSIDE-INDUCED TOXICITY IN MAMMALIAN CELL CULTURES.

M. Kong[1], H. Ha[1], H. Kim[2], G. Ko[1], S. Yoo[1], D. Lee[1], M. Kang[1], H. Jeong[2] and T. Jeong[1]. [1]*Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea and* [2]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea.*

A possible role of metabolism by intestinal bacteria in geniposide-induced toxicity was investigated in mammalian cell cultures. Following an incubation of geniposide with intestinal bacteria for 24 h, it was estimated that geniposide was metabolized to genipin in the bacterial culture media. When the toxicity was compared in the HepG2 cell lines, genipin was more toxic than the parent geniposide, indicating that metabolic activation might be required in geniposide-induced toxicity. After geniposide was incubated with intestinal bacteria for 24 h, cytotoxicity was highly increased when compared to geniposide control. The present results indicate that the current system might be useful for assessing the possible role of bacterial metabolism in mammalian cell cultures. (Supported by a grant (09172KFDA996) from Korea Food & Drug Administration in 2010.)

PS 1828 METABOLIC CONVERSION OF AFLATOXIN B2 TO AFLATOXIN B1 IN DUCKS.

A. Poapolathep[1], S. Poapolathep[1], S. Isariyodom[3], K. Imsilp[1], N. Klangkaew[1], Y. Sugita-Konishi[4] and S. Kumagai[2]. [1]*Department of Pharmacology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand,* [2]*Research Center for Food Safety, University of Tokyo, Tokyo, Japan,* [3]*Department of Animal Husbandry, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand and* [4]*Division of Microbiology, National Institute of Health Sciences, Tokyo, Japan.*

Our previous research revealed that AFB2 can be converted to AFB1 by using post-mitochondrial (S-9) fraction of liver from six different animal species with different magnitudes. Among those, duck livers were more susceptible to the AFB2-to-AFB1 conversion. This study was aimed to investigate this AFB2-to-AFB1 conversion in whole ducks. We, thus, administered AFB2 to ducks either intravenously (IV) or orally (PO) at a dosage of 1 mg/kg body weight. Plasma and liver were collected and analyzed for both AFB2 and AFB1 using liquid chromatography/tandem mass spectrometry (LC/MS/MS). The AFB2 was detected in plasma for up to 12h and 8h whereas AFB1 was up to 1h and 30 min after IV and PO, respectively. Both aflatoxins were also found in duck livers. This in vivo study confirms that AFB2 can be metabolized to AFB1 in ducks as those found in the in vitro study.

PS 1829 THIOETHER METABOLITES OF 3, 4-METHYLENEDIOXYMETHAMPHETAMINE ARE POTENT INHIBITORS OF THE HUMAN SEROTONIN TRANSPORTER (HSERT).

L. E. Lizarraga, J. Herndon, S. S. Lau and T. J. Monks. *Pharmacology/Toxicology, University of Arizona-College of Pharmacy, Tucson, AZ.*

3,4-Methylenedioxymethamphetamine (MDMA, ecstacy) is a ring substituted amphetamine derivative with potent stimulant properties. MDMA long-term serotonergic neurotoxicity manifests as prolonged depletion in serotonin (5-HT) and structural damage to 5-HT axons. The former effect is likely mediated via the ability of MDMA to inhibit 5-HT uptake via the 5-HT transporter (SERT), since fluoxetine, a selective 5-HT reuptake inhibitor, protects against MDMA-induced ROS generation and neurotoxicity. The basis for the structural damage remains unclear. Metabolism of MDMA to N-methyl-α-methyldopamine (N-Me-α-MeDA), followed by oxidation and adduction of the corresponsing ortho-quinone with glutathione (GSH) leads to the formation of 5-(glutathion-S-yl)-N-Me-α-MeDA and 2,5-bis(GSyl)-N-Me-α-MeDA. Thioether conjugates of N-Me-α-MeDA are neurotoxic, however, their mechanism of action remains unclear. We now investigated the effect of GSH and N-acetylcysteine (NAC) conjugates of N-Me-α-MeDA on SERT function. Human embryonic kidney cells (HEK) were transiently transfected with hSERT and incubated with [3H]-5-HT to measure transport via SERT. We confirmed expression of SERT protein by western blot analysis of HEK-SERT. The cellular uptake of [3H]-5-HT into hSERT-expressing cells was rapid and continued to accumulate, reaching maximal uptake after 50 minutes. Km and Vmax values for specific [3H]5-HT uptake were determined to be 7.9 μM and 0.23 μM/min, respectively. The inhibitory effect of MDMA metabolites on SERT function was determined by measuring uptake of [3H]5-HT with varying concentrations of the metabolites. N-Me-α-MeDA thioether metabolites inhibited [3H]5-HT uptake into HEK-SERT cells, and to a greater extend than the parent drug, MDMA. The results are consistent with the hypothesis that MDMA-induced serotonergic neurotoxicity involves metabolism-mediated modulation of SERT function. We are currently investigating MDMA effects in SERT-KO rats to further address the role of SERT in MDMA-mediated neurotoxicity.

PS 1830 METABOLISM OF 2, 2', 3, 3', 6, 6'-HEXACHLOROBIPHENYL (PCB136) IN PRECISION-CUT RAT LIVER SLICES.

X. Wu[1], K. Dammanahalli[2], M. Duffel[2] and H. Lehmler[1]. [1]*Occupational and Environmental Health, The University of Iowa, Iowa City, IA and* [2]*Medicinal and Natural Products Chemistry, The University of Iowa, Iowa City, IA.*

Biotransformation of 2,2',3,3',6,6'-hexachlorobiphenyl (PCB136) may contribute to the developmental toxicity of polychlorinated biphenyls (PCBs) through the formation of neurotoxic metabolites. Here we investigate the hypothesis that PCB136 can be enantioselectively metabolized to hydroxylated PCB136 (OH-PCB136) metabolites in precision-cut rat liver slices. Liver tissue slices (250 μm thickness, 8 mm diameter) were obtained from 8-week old female or male Sprague-Dawley rats treated for three or four consecutive days with either Phenobarbital (PB, CYP2B inducer) or dexamethasone (DEX, CYP3A inducer). Tissue slices were incubated for 2 h with Krebs-Henseleit buffer (pH 7.4) containing 5 μM PCB136. Lactate dehydrogenase release from the tissue slices to the medium was 12.8% indicating the viability of the liver slices during the incubation. Levels of PCB136 and its hydroxylated metabolites in tissue slices and medium were determined by gas chromatography using a 63Ni μ-ECD detector. PCB136 levels in medium were 3~7 times higher compared to tissue slice levels after incubation. Less than 10% of PCB136 was biotransformed to its hydroxylated metabolites. Levels of 5-OH PCB136 were 2-times higher in tissue slices from PB- versus DEX-treated rats. The metabolite pattern in PB- and DEX-treated rats was similar and followed the rank order 5-OH-PCB136 >> 4-OH-PCB136 > 4,5-diOH-PCB136. The enantiomeric fraction (EF) of PCB136 in medium samples was 0.50, while the EF value in the liver slices (EF = 0.48 to 0.49) displayed a slight enrichment of (-)-PCB136 after 2 h incubation. No gender-specific differences in the metabolite profile or the EF values were observed. Overall, the metabolite pattern of PCB136 in rat liver slices was

comparable to in vivo disposition studies, which suggests that precision-cut tissue slices are a useful model for studying the (enantioselective) metabolism of PCB136 in different tissues (supported by ES05605, ES013661 and ES017425).

PS 1831 STRUCTURAL CHARACTERIZATION OF O-METHYLATED-CATECHOL METABOLITE OF BENZO[A]PYRENE-7, 8-DIONE IN THREE HUMAN LUNG CELLS.

M. Huang, L. Zhang, I. A. Blair and T. M. Penning. *Centers of Excellence in Environmental Toxicology and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania, Philadelphia, PA.*

Benzo[a]pyrene (B[a]P), a representative polycyclic aromatic hydrocarbon, is a ubiquitous environmental pollutant occurring in tobacco smoke and residues of fossil fuel combustion. Metabolic activation of the proximate carcinogen B[a]P-7,8-trans-dihydrodiol by aldo-keto reductases (AKRs) leads to B[a]P-7,8-dione that is redox-active and generates reactive oxygen species resulting in oxidative DNA damage in human lung cells. O-methylation of the corresponding catechol by cactechol-O-methyltransferase (COMT) is predicted as one pathway for detoxification of B[a]P-7,8-dione. We investigated the occurrence of this pathway in human bronchoalveolar H358 cells, human lung adenocarcinoma A549 cells, and immortalized human bronchial epithelial HBEC-KT cells following treatment of B[a]P-7,8-dione for 24 hours. After acidification of the culture medium, a single o-methyl-B[a]P-7,8-catechol product was detected in the organic phase of medium from each cell line using HPLC-UV and LC-MS/MS. An authentic metabolite standard was subsequently produced by enzymatic synthesis and purified by semi-preparative HPLC and characterized by [1H]- NMR. The definite structure of the cellular metabolite was identified to be o-8-methyl-B[a]P-7,8-catechol. It is concluded that human COMT may play a critical role in the detoxification of B[a]P-7,8-dione in lung cells [Supported by P30-ES013508 and 1R01-CA-39504 awarded to TMP].

PS 1832 A MEANS TO STUDY CRITICAL ADDUCT PROTEIN TARGETS: COMPARISON OF TOXIC NAPHTHALENE AND NON-TOXIC DIETHYL MALEATE SHOWS EQUIVALENT *IN VIVO* COVALENT BINDING AND GLUTATHIONE DEPLETION IN AIRWAY EPITHELIUM.

D. Krawiec[1], D. Morin[1], L. Van Winkle[2] and A. Buckpitt[1]. [1]*Molecular Biosciences, UC Davis, Davis, CA and* [2]*Anatomy Physiology and Cell Biology, UC Davis, Davis, CA.*

Reactive metabolites have the potential to bind covalently to cellular macromolecules (protein and/or DNA) and these interactions have been associated with the carcinogenic and toxic actions of some chemicals. Not all reactive metabolite protein binding results in cytotoxicity. This suggests that either overall adduct levels are not important or there are critical proteins that are adducted by cytotoxic reactive metabolites whereas these are not modified by reactive, non-cytotoxic metabolites. To test the hypothesis that critical protein adducts can be discriminated from non-critical protein targets, we have evaluated the formation and persistence of reactive metabolite protein adducts from naphthalene, which causes necrosis of Clara cells, and diethyl maleate which produces transient swelling of airway epithelial cells but in no necrosis (Phimister et al., JPET 314: 506, 2005). In vivo comparison of protein binding in airway epithelium for both NA (300 mg/kg) and DEM (1000 mg/kg), doses which produce equivalent depletion of airway epithelial glutathione levels, have shown an average covalent binding which is identical for the two compounds: 1.42 ± 0.13 nmoles/mg protein for NA and 1.43 ± 0.15 nmoles/mg protein for DEM between 1 and 8 hours post-dose. Adducts are stable within the measured time frame, indicating that the differences in cellular response are not related to differential persistence of adducts on protein. GSH depletion was also similar for the two compounds at the doses used in the protein adduct studies: 32.8 ± 17.3 percent control for NA and 41.3 ± 17.5 percent control for DEM between 1 and 4 hours. Identification of proteins targeted by DEM and comparison to known NA adducts provides a novel approach to identifying critical and non-critical protein adducts in the lung. Supported by NIEHS 04311 and 04699

PS 1833 EFFECT OF DDT ON TESTOSTERONE BIOTRANSFORMATION BY RAT BRAIN MICROSOMES.

A. Sierra-Santoyo[1], O. Horacio[2] and M. L. Lopez-Gonzalez[1]. [1]*Departamento de Toxicologia, CINVESTAV-IPN, Mexico, D.F., Mexico and* [2]*Departamento de Nefrologia, Inc., Mexico, D.F., Mexico.*

Dichlorodiphenyltrichloroethane (DDT) is a liver cytochrome P-450 (CYP) inducer and an endocrine disruptor. Information about the effect of DDT on CYP expression in extrahepatic tissues is limited. The objective of this study was to determine the effect of DDT on CYP-dependent testosterone hydroxylase activities by rat brain microsomes. Wistar adult male rats were administered with an oral dose of DDT technical grade (0.1, 1, 5, 10 and 100 mg/kg) dissolved in corn oil. Animals were sacrificed 24 h later and brain was removed and processed to obtain microsomes. Brain microsomes were incubated with [4-^{14}C]-testosterone and metabolites were separated by thin layer chromatography and quantified by radio scanning and CYP2B1/2, 2A, 2C11 and 3A2 levels were determined by Western blot analysis. DDT altered testosterone biotransformation and modified the profile of metabolites. A significant reduction on 2α-hydroxytestosterone (OHT), 6α-OHT and 7α-OHT was observed at all doses. At the doses of 0.1 and 1 mg/kg a significant decrease on 15α-OHT, 6β-OHT and androstenedione formation was detected. At the dose of 100 mg/kg the 6-dehydrotestosterone formation increased 2-fold while at the doses of 5 and 10 mg/kg 16α-OHT formation increased more than 2-fold. CYP2A and 2B1/2 levels increased at the doses from 0.1 to 5 mg/kg and decreased at the two highest doses respect to the control group. CYP2C11 levels decreased in a dose-dependent manner at all doses. These results indicate that a single dose of DDT modifies brain CYP-dependent testosterone-hydroxylating activities and represent another endocrine disruption mechanism.

PS 1834 TWO MECHANISMS INVOLVED IN ECSTASY-INDUCED HEPATOTOXICITY.

I. Antolino Lobo[1, 2], W. Strach[1], D. A. Fiechter[1], I. S. Ludwig[1], M. van den Berg[1], J. Meulenbelt[1, 2] and M. van Duursen[1]. [1]*Endocrine Toxicology, IRAS, Utrecht, Netherlands and* [2]*National Poisons Information Centre, National Institute for Public Health and the Environment., Bilthoven, Netherlands.*

3,4-methylenedioxymethamphetamine (Ecstasy, MDMA) is a widely used recreational drug, which is known to induce hepatotoxicity in humans. Yet, the mechanism of MDMA-induced hepatotoxicity is still unclear. We have investigated to role of MDMA metabolism and immunological responses *in vitro*, two suggested pathways which could lead to MDMA-induced liver injury. To study the role of specific metabolic pathways in MDMA toxicity, a human liver epithelial (THLE) cell line transfected with a single cytochrome P450 enzyme (CYP450) was used. We observed major MDMA-induced toxic events after CYP3A4 and CYP2D6-mediated metabolism with a reduced cell viability of 50% and 25% at 4mM MDMA, respectively. In addition, MDMA exposure resulted in a 40% decrease of GSH level in THLE-CYP3A4, but not THLE-CYP2D6 cells. This indicates that CYP3A4-formed MDMA metabolites and subsequent GSH depletion play a key role in MDMA–induced cytotoxicity. To evaluate the possible contribution of immune responses in MDMA-induced liver toxicity, we established a co-culture model with a classical human liver cell line (HepG2) and a human monocyte cell line (THP-1). When cell viability of mono-cultured HepG2 cells was compared with co-cultured HepG2 and THP-1 cells, an increased cell viability of 10% at 4 mM MDMA exposure was observed in HepG2 cells. This indicates a moderately protective role of monocytes on liver cells upon MDMA exposure. Cytokine levels (TNF-α, IL-1β) and the chemokine IL-8 are being evaluated to clarify these findings. Taken together, these data suggest that both CYP3A4-mediated metabolism and immune responses can affect MDMA-induced cytotoxicity in liver cells *in vitro*. Studies are currently being performed to elucidate the suggested mechanisms and to determine the clinical relevance of these findings.

PS 1835 TOXICITY OF AMINONITRILES IN MALE SPRAGUE-DAWLEY RATS.

M. Y. Farooqui and T. Lacy. *Biology, University of Texas Pan American, Edinburg, TX.*

Aminonitriles (AN) are especially potent representatives of bifunctional compounds. Importance of Amino moiety for drug design is hard to overestimate due to a set of intrinsic properties and nitrile functional group can be involved into hydrogen-bonding and can be selectively transformed to primary amine, aldehyde, ketone, or carboxylic acid (hydrolysis). Some toxicity symptoms of AN appear in the literature. We have studied toxicities and metabolism of 4 AN namely aminoacetonitrile, β-aminopropionitrile, N,N- dmethylaminopropionitrile and β,β-Imminodipropionitrile in male Sprague-Dawley rats using 0.25 LD50 oral doses. Following treatment rats were observed for visible signs of toxicity and concentrations of cyanide, thiocyanate, cyanoacetic acid and glutathione were determined at various time intervals. All of the AN studied produced significant cholinomimetic effects including vasodilation in ears, bloated abdomens, disorientation, ataxia and visual impairment. Additional significant effects observed were severe cerebral hemorrhage and urinary retention in the bladders. The concentration of cyanide increased 4 to 7 fold in plasma and organs. The concentrations of thiocyanate increased 2-4 fold in plasma and urine. Levels of glutathione significantly decreased 30-60% of controls in organs. The concentrations of another metabolite

cyanoacetic acid increased 2-3 fold in plasma and urine. Urinalysis of rats showed a significant proteinuria. We also studied the role of vehicles in the toxicity of AN. The effects were variable in various organs. Among the vehicles studied corn oil, olive oil and safflower oil had a significant effect in increasing or decreasing the levels of metabolites studied in different organs. [Supported by Grant No. SO6RR08038 from the Minority Biomedical Research Support Program of the National Institutes of Health]

PS 1836 DRUGS INTERACTING WITH TRICHLOROETHYLENE METABOLISM IN RAT AND HUMAN.

M. Cheikh-Rouhou[2] and S. Haddad[1]. [1]*Environmental and Occupational Health, Université de Montréal, Montréal, QC, Canada and* [2]*Biological Sciences, TOXEN, Université du Québec à Montréal, Montréal, QC, Canada.*

We recently studied the impact of 14 commonly used drugs on the metabolism of Trichloroethylene (TCE) in rat hepatocytes and identified that 5 altered the kinetics of formation of trichloroethanol (TCOH) and trichloroacetic acid (TCA). The objective of this study were i) to determine if the same interactions are observable in human hepatocytes, and ii) to characterize the stronger interactions using microsomal assays. First, the interacting drugs have been identified in vitro by measuring the metabolite formation rates in suspensions of human hepatocytes in the presence and absence of each of the drugs in closed vials. The concentrations of TCE and its metabolites TCOH and TCA were measured GC-MS. Similar to observations in rat hepatocytes, acetyl salicylic acid and naproxen increased significantly TCA and TCOH levels, while the significant decreased levels in both metabolites were not observed by the same drugs (rat: valproic acid, acetaminophen and gliclazide; and human: carbamazepine and erythromycine). Drugs decreasing only TCOH levels were different in both species: sulfasalazine and valproic acid in humans, and erythromycine in rat. Decreases in TCA only were observed with gliclazide in humans, and with cimetidine, diclofenac and amoxicillin in rats. Although metabolites levels in hepatocytes were shown to increase in the presence of salicylic acid and decrease with acetaminophen, no effect were were detected using microsomes. Results from characterization in rats shows that gliclazide (Ki= 566.7μM, TCOH formation) and valproic acid (Ki= 624.2 μM for TCA formation and Ki= 682 μM for TCOH formation) altered TCE microsomal oxidation by mixed partial inhibition. And naproxen partially competitively inhibited TCOH glucuronidation (Ki = 294.8 μM). Combined in vivo rat exposures of TCE with to the identified stronger interacting drug are underway and in vitro–in vivo extrapolations of interactions will be validated with PBPK modeling to ultimately enable predictions of interactions in humans.

PS 1837 HUMAN DIOXIN-INDUCIBLE CYTOCHROME P450, CYP2S1, METABOLIZES CYCLOOXYGENASE – AND LIPOXYGENASE – DERIVED EICOSANOIDS.

P. H. Bui[1, 2, 3], S. Imaizumi[4], S. Beedanagari[1, 2], S. T. Reddy[4] and O. Hankinson[1, 2, 3]. [1]*Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, CA,* [2]*Molecular Toxicology Interdepartmental Program, University of California, Los Angeles, Los Angeles, CA,* [3]*Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles, CA and* [4]*Department of Molecular and Medical Pharmacology, University of California, Los Angeles, Los Angeles, CA.*

Human CYP2S1 is a recently described dioxin-inducible cytochrome P450 which can oxidize a number of carcinogens via the peroxide shunt. To further characterize the biological function of this enzyme, we investigated whether it can metabolize cyclooxygenase (COX) and lipoxygenase (LOX) derived lipid peroxides in a NADPH-independent fashion. Human CYP2S1 metabolizes prostaglandin G2 (PGG2) (Km = 0.267±0.072 μM) into several products including 12S-hydroxy-5Z,8E,10E-heptadecatrienoic acid (12-HHT). It also metabolizes prostaglandin H2 (PGH2) (Km=11.7 ± 2.8 μM) into malondialdehyde (MDA), 12-HHT, and thromboxane A2 (TXA2). The turnover to 12-HHT by human CYP2S1 (1.59±0.04 min^{-1}) is 40-fold higher than that of TXA2 (0.04 min^{-1}). In addition to PGG2 and PGH2 metabolism, human CYP2S1 efficiently metabolizes the hydroperoxyeicosatetraenoic acids (HpETE), 5S-, 12S-, 15S- HpETEs and 13S-hydroperoxyoctadecadienoic acid (13S-HpODE) into 5-oxo-eicosatetraenoic acid (5-oxoETE) (turnover = 16.7 ± 0.3 min^{-1}), 12-oxo-eicosatetraenoic acid 1(12-oxoETE) (11.5 ± 0.9 min^{-1}), 15-oxo-eicosatetraenoic acid (15-oxoETE) (16.9 ± 0.8 min^{-1}), and 13-octadecadienoic acid (13-oxoODE) (20.2±0.9 min^{-1}), respectively. Other P450s such as CYP1A1, 1A2, 1B1, and 3A4 carried out similar conversions, but at slower rates. The fatty acid hydroperoxides were also converted by human CYP2S1 to several epoxy alcohols. Our data indicate that fatty acid endoperoxides and hydroperoxides represent endogenous substrates of CYP2S1 and suggest that the enzyme CYP2S1 may play an important role in the inflammatory process since some of the products that CYP2S1 produces play important role in inflammation.

PS 1838 CYTOCHROME P450'S VARY IN THEIR ABILITY TO GENERATE REACTIVE OXYGEN SPECIES.

V. Mishin[1], D. E. Heck[2], D. L. Laskin[1] and J. D. Laskin[3]. [1]*Pharmacology & Toxicology, Rutgers University, Piscataway, NJ,* [2]*Environmental Health Sciences, New York Medical College, Valhalla, NY and* [3]*Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

In the presence of NAD(P)H, the microsomal enzymes are able to generate hydrogen peroxide as a result of the non-productive utilization of reducing equivalents, a reaction known as 'uncoupling'. The precise origin of hydrogen peroxide in this reaction is not known and may be generated directly or indirectly via the formation of superoxide anion. Hydrogen peroxide generated by this process can form highly toxic hydroxyl radicals in the presence of redox active transition metals; depending on the cytochrome P450's present in tissues, this may compromise cellular functioning and contribute to tissue injury. In present studies we quantified the relative 'uncoupling' activity of different human recombinant cytochrome P450's using a modified end-point Amplex Red/horse radish peroxidase assay. The complex of human recombinant NADPH-cytochrome P450 reductase and cytochrome b5 (in the absence of cytochrome P450 enzymes) generated low, but detectable amounts of hydrogen peroxide (0.05-0.1 nmoles hydrogen peroxide/min/100 Units of reductase). Significantly higher activity was found when NADPH cytochrome P450 reductase/b5 reductase was coexpressed with various cytochrome P450's. Activities ranged from 6.0 nmoles hydrogen peroxide formed/nmole P450 to 0.4 nmole/nmole P450 with CYP1A1 as the most active, followed CYP2D6, CYP3A4, CYP4A11, CYP2E1, CYP1A2 and CYP2C. Substrate binding is thought to facilitate oxygen activation by the cytochrome P450 enzymes. In contrast, we found that the hydrogen peroxide generating activity of the recombinant CYP's did not require the addition of any cytochrome P450 substrates. Since CYP3A4/5 and CYP2C8/9/18/19 represent up to 80% of total cytochrome P450 in the liver, these enzymes most likely represent a major source of hydrogen peroxide in human liver microsomes. Supported by AR055073, NS072097, ES005022, GM034310, ES004738 and CA132624.

PS 1839 BACKGROUND LEVELS OF THE TRYPTOPHAN PHOTOPRODUCT 6-FORMYLINDOLO[3, 2-b]CARBAZOLE (FICZ) DETERMINE THE OUTCOME OF *IN VITRO* BIOASSAYS FOR AH-RECEPTOR ACTIVATION.

U. Rannug[1], E. Wincent[1, 2], A. Mohammadi Bardbori[2], T. Alsberg[3] and A. Rannug[2]. [1]*Department of Genetics, Microbiology and Toxicology, Stockholm University, Stockholm, Sweden,* [2]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and* [3]*Department of Applied Environmental Science, Stockholm University, Stockholm, Sweden.*

6-Formylindolo[3,2-*b*]carbazole (FICZ) is formed when tryptophan containing cell culture media are exposed to UV radiation or visible light. This substance binds to the aryl hydrocarbon receptor (AHR) with very high affinity and is a claimed endogenous AHR ligand (Rannug et al., J Biol Chem, 262, 1987, 15422-27). FICZ is a potent inducer of CYP1 enzymes and an excellent substrate for CYP1A1 and may thus interfere with *in vitro* bioassays for AHR activation and toxicity of AHR dependent xenobiotics.

Our earlier analyses employing HPLC/MS demonstrated that Dulbecco's Modified Eagle Medium (DMEM) exposed to light contained FICZ at concentrations up to 8 pM (Öberg et al., Tox Sci, 85, 2005, 935-43). Here we describe analyses of light-protected commercial media and again we could detect FICZ, but at concentrations around 0.2 pM. The biological consequences of these low background concentrations of FICZ were analyzed in HaCaT cells grown in DMEM. The cells were exposed to H_2O_2 or 3'-methoxy-4'-nitroflavone (MNF), two substances reported to have AHR activating properties. Both substances increased CYP1A1 mRNA expression and 7-ethoxyresorufin *O*-deethylase activity. Interestingly, these effects were only seen in commercial medium and not in DMEM prepared with purified tryptophan and hence lacking FICZ. When FICZ was added to this medium at a concentration of 0.1 pM the CYP1A1 activation by MNF was regained. Both substances also inhibited the CYP1A1 catalyzed turnover of FICZ, and if present together with FICZ, they prolonged the otherwise transient AHR-mediated CYP1A1 induction. These data show that background levels of FICZ in commercial media are high enough to explain activation of the AHR and that erroneous conclusions could be drawn from *in vitro* bioassays in screening for AHR activators.

PS 1840 CYTOCHROME P450 CYP2AA, CYP3A, AND PREGNANE X RECEPTOR IN ZEBRAFISH (*DANIO RERIO*): EXPRESSION IN RESPONSE TO POTENTIAL AGONISTS FOR PXR.

A. Kubota[1], A. Bainy[2, 1], M. Siebert[2], B. Woodin[1], J. Goldstone[1] and J. Stegeman[1]. [1]*Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA and* [2]*Departamento de Bioquímica, Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil.*

Understanding the roles of cytochrome P450 (CYP) in zebrafish is important to the use of this non-mammalian model in toxicological, pharmacological and carcinogenesis research. Here we assessed responses to potential agonists for zebrafish pregnane X receptor (PXR), to establish target genes in this species. Adult zebrafish were injected i.p. with carrier (DMSO) or potential agonists for the PXR. Three days after the injection, the liver tissue was excised, flash frozen in LN_2, and stored at -80 °C until analyzed by real time PCR. Expression of newly identified CYP2AA1 and CYP2AA2, as well as CYP3A65 and PXR was examined. Zebrafish CYP2AA1 mRNA is highly expressed in kidney, while CYP2AA2 is expressed in liver, kidney, spleen, ovary and intestine, suggesting differing roles in biotransformation of exogenous and/or endogenous compounds. CYP2AA1 expression in liver was increased by 5-pregnen-3β-ol-20-one (pregnenolone), while CYP2AA2 was induced by all potential PXR agonists examined, including pregnenolone, pregnenolone-16α-carbonitrile (PCN), phenobarbital (PB) and 1,4-bis-[2-(3,5-dichloropyridyloxy)]-benzene (TCPOBOP). CYP2AA2 was also induced by 2,2,4,4,5,5-hexachlorobiphenyl (PCB153). Similar to CYP2AA2, expression of CYP3A65 and PXR was significantly increased by most chemicals tested. Importantly, combined treatment with PCN and PCB153 induced expression of CYP2AA2, CYP3A65 and PXR much greater than single administration with each chemical. Thus, treatment with PCN+PCB153 in combination gave an additive effect on the CYP2AA2, CYP3A65 and PXR induction in liver. The induction of CYP2 genes is the first concrete evidence for a PB-type response in fish, and as zebrafish lacks the constitutive androstane receptor, suggests involvement of PXR.

PS 1841 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATIONS OF CYP1A1 GENE BY LEAD IN HUMAN HEPATOMA HEPG2 CELL.

H. M. Korashy[1] and A. O. El-Kadi[2]. [1]*Pharmacology & Toxicology, King Saud University, Riyadh, Saudi Arabia and* [2]*Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.*

Co-contamination with complex mixtures of heavy metals, such as lead (Pb^{2+}) and polycyclic aromatic hydrocarbons (PAHs), such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) may disrupt the coordinated regulation of the carcinogen activating enzyme cytochrome P450 1A1 (CYP1A1). Yet the mechanisms involved remain unknown. Therefore, the aims of the current study were to a) investigate the effect of co-exposure to Pb^{2+} and TCDD on the expression of the CYP1A1 in human hepatoma (HepG2) cells and b) explore the molecular mechanisms involved. For this purpose, HepG2 cells were incubated with TCDD (1 nM) in the presence and absence of increasing concentrations of Pb^{2+} (10, 25 and 100 μM). Cell viability was determined using the MTT assay. CYP1A1 mRNA, protein, and activity levels were measured using real-time PCR, Western blot, and 7-ethoxyresorufin O-deethylase analyses, respectively. Our results showed that Pb^{2+} significantly inhibited the TCDD-mediated induction of CYP1A1 at the mRNA, protein, and catalytic activity levels. Mechanistically, co-exposure to Pb^{2+} and TCDD significantly decreased the TCDD-mediated induction of AhR-dependent luciferase reporter gene expression. Importantly, Pb^{2+} increased the expression of heme oxygenase-1 (HO-1), a rate limiting enzyme in heme degradation, which coincided with further decrease in the CYP1A1 catalytic activity levels. In addition, pretreatment with a HO-1 competitive inhibitor, tin mesoporphyrin, a heme precursor, hemin, or transfecting the HepG2 cells with siRNA for HO-1, significantly restored the inhibition of TCDD-mediated induction of CYP1A1 catalytic activity. In conclusion, we demonstrate that Pb^{2+} down-regulates the expression of CYP1A1 at the transcriptional and posttranscriptional levels in HepG2 cells through a HO-1-dependent mechanism.

PS 1842 EFFECTS OF THE STANDARDIZED EXTRACT OF *CENTELLA ASIATICA* (ECA 233) ON HUMAN CYTOCHROME P450.

S. T. Lawanprasert, N. Niwattisaiwong, W. Warisnoicharoen, P. Seeka, T. Winitthana and M. H. Tantisira. *Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand.* Sponsor: L. Robertson.

The standardized extract of Centella asiatica (ECa 233) was shown to exhibit learning and memory enhancing effects. Beside toxicity testing data, safety data regarding drug interaction is also required. The purpose of this study was to investigate effects of ECa 233 on major human cytochrome P450 (CYP) by using recombinant human CYP in the in vitro study. The method was verified by using the selective inhibitor of the individual CYP isoform. The results showed that ECa 233 inhibited CYP3A4 (IC50 = 210.98 μg/ml), CYP2C19 (IC50 = 365.18 μg/ml) and CYP2B6 (IC50 = 871.14 μg/ml). ECa 233 did not affect CYP1A2, CYP2C9, CYP2D6 and CYP2E1. Inhibition of ECa233 on CYP3A4 was shown to be mechanism-based as well as noncompetitive inhibition with a Ki value of 769 μM. This finding provided a preliminary information that ECa 233 possessed a potential effect to interact with other medicines that are metabolized by CYP3A4, CYP2C19 and CYP2B6. Further study is needed to investigate whether or not this effect is clinically significant.

PS 1843 BUTADIENE EPOXIDE METABOLITES INHIBIT CYP2E1 ACTIVITY.

J. H. Hartman[1], G. P. Miller[1] and G. Boysen[2]. [1]*Biochemistry, University of Arkansas for Medical Sciences, Little Rock, AR and* [2]*Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR.*

1,3-Butadiene (BD) is a known rodent and human carcinogen metabolized mainly by CYP2E1 to three epoxides, 1,2-epoxy-3-butene (EB), 1,2:3,4-diepoxybutane (DEB) and 1,2-epoxy-3,4-butanediol (EB-diol). The metabolites vary up to 200-fold in their mutagenic potency, with DEB being the most mutagenic. We recently showed that formation of BD-derived epoxides is species and concentration dependent and that formation of some epoxides saturate at higher exposures. It is hypothesized that BD or its metabolites inhibit their own metabolism. To determine which metabolite is most likely responsible for saturation, the inhibitory potencies of BD metabolites (EB, DEB and BD-diol) on human CYP2E1 activity for 4-nitrophenol hydroxylation were determined using reconstituted human CYP2E1 or HLM 150. In addition, known CYP2E1 inhibitors such as 4-methylpyrazole (4-MP) and dimethylnitrosamine (DMN) were included to put the in vitro inhibition results into perspective. Of the BD metabolites studied, EB was the strongest inhibitor with an IC50 of 110 μM compared to 1700 μM and 6600 μM for BD diol and DEB, respectively. Compared to the known inhibitors, EB's inhibitor potency is somewhat between 4-MP (IC50 = 1.8 μM) and DMN (IC50 = 230 μM). All three BD metabolites inhibit CYP2E1 activity through a simple competitive mechanism. Among the BD metabolites effects seem to be influenced by the presence and location of polar groups. To further examine this notion, the investigation was extended to structural analogs of the BD metabolites, including propylene oxide (PO), 1-butanol, 2-butanol, 1,2-butanediol, 1,4-butanediol, and 1,2,4-butanetriol. Those results demonstrated preference of CYP2E1 toward the type and location of polar and hydrophobic structural elements. Ultimately, our findings suggest that in complex exposure situations, interactions between compounds will significantly alter metabolism of individual carcinogens, which should be incorporated in mechanism-based risk assessments of mixtures.

PS 1844 EFFECTS OF HERBAL EXTRACTS ON CYTOCHROME P450-ASSOCIATED MONOOXYGENASE ACTIVITIES IN ENRICHED RAT LIVER MICROSOMES.

D. Lee, M. Kong, G. Ko, S. Yoo, H. Ha, T. Jeong and M. Kang. *Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea.*

To characterize possible herb-drug interactions, effects of herbal extracts on cytochrome P450 (CYP)-associated monooxygenase activities were investigated in certain CYP-enriched rat liver microsomes. To enrich CYP enzymes, either 3-methylcholanthrene (3-MC, 40 mg/kg, ip, for 3 days), phenobarbital (PB, 80 mg/kg, ip, for 3 days), dexamethasone (DEX, 50 mg/kg, ip, for 3 days), or acetone (ACE, 5 ml/kg, po, once for 2 days) were used. Following treatment with inducers, liver microsomes were isolated and used for assaying ethoxyresorufin O-deethylase (EROD) with 3-MC microsomes for CYP 1A, pentoxyresorufin O-depentylase (PROD) with PB microsomes for CYP 2B, erythromycin N-demethylase (ERDM) with DEX microsomes for CYP 3A, and p-nitrophenol hydroxylase (PNPH) with ACE microsomes for CYP 2E1. The concentration of each extracts was ranged 3.3 to 300 μg/ml with 5 concentrations for determining the 50% inhibitory concentration (IC_{50}). For EROD activity, inhibitory effects of ethanol extracts from *Auklandia lappa* (IC_{50}, 25.9 μg/ml) and *Artemisia capillaris* (IC_{50}, 18.0 μg/ml) were considerable. Effects on PROD were profound for ethanol extracts of *Auklandia lappa* (IC_{50}, 6.0 μg/ml) and *Atractylodes japonica* (IC_{50}, 5.0 μg/ml). Meanwhile, the extracts from *Zizyphus jujube* and *Artemisia capillaris* inhibited ERDM activity among others. Effects of the extracts tested in PNPH were marginal. The present results suggested that the ethanol extracts of some herbs should be studied further to confirm the possible herb-drug interactions in vivo. [Supported by a grant from the Korean Food and Drug Administration for Studies on Standardization of Herbal Medicine (2010).]

PS 1845 IS THE PROMISCUITY OF THE ARYL HYDROCARBON RECEPTOR A MYTH?

E. Wincent[1, 2], J. Bengtsson[2], U. Rannug[2] and A. Rannug[1]. [1]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and* [2]*Department of Genetics, Microbiology and Toxicology, Stockholm University, Stockholm, Sweden.*

Compounds with little structural resemblance to dioxins, including several oxidants and inhibitors of cytochrome P4501A1 (CYP1A1), activate the aryl hydrocarbon receptor (AHR) pathway. With such a wide variety of known activators, the AHR is generally regarded as a promiscuous receptor. Instead, we suggest that many agents, exemplified by ultraviolet B rays (UVB), hydrogen peroxide (H_2O_2) and 3′-methoxy-4′-nitroflavone (MNF), are not true AHR agonists but activate the receptor in an indirect manner by affecting the formation and/or clearance of the endogenous high affinity AHR ligand 6-formylindolo[3,2-b]carbazole (FICZ). In the AHR signaling pathway, the CYP1 enzymes participate in an autoregulatory feedback that maintains the steady-state concentrations of FICZ at low levels. We claim that disruption of this highly efficient feedback mechanism will have biological consequences that include induction of AHR regulated genes. To demonstrate the impact of CYP1A1 dependent regulation of AhR signaling, dose-response and time course studies were performed using qRT-PCR and CYP1A1 enzyme activity measurements in immortalized and primary human keratinocytes (HaCaT, HEKa). Exposure to UVB, H_2O_2 or MNF resulted in increased CYP1A1 enzyme activity as measured with 7-ethoxyresorufin as a substrate at 24h and 48h after start of exposure. However, within the early phase after exposure start, the transcription of the CYP1A1 gene and the CYP1A1 enzyme activity were strongly impaired. Thus, these agents can both activate the AHR pathway and act as efficient AHR antagonists. Furthermore, HPLC analyzes of intracellular levels of FICZ showed that all agents efficiently delay the metabolic degradation of FICZ.
These results clearly demonstrate that inhibition of CYP1 dependent metabolism disturbs the crucial feedback regulation of an endogenous activator. Since FICZ is a bioactive compound having also potent AHR independent effects, the biological significance of CYP1A1 inhibitors should be investigated further.

PS 1846 *IN VITRO* METABOLISM OF DI(2-ETHYLHEXYL) PHTHALATE (DEHP) BY VARIOUS HUMAN AND RAT ORGAN MICROSOMES, S9, AND CYP ISOFORMS.

J. Campbell, K. Choi, H. Joo, R. Clewell and H. Clewell. *The Hamner Institutes, Research Triangle Park, NC.*

We have investigated the metabolism of DEHP in human and rat using S9, microsomal (MIC), and cytosolic (Cytol) fractions of various organs and CYP isoforms.. In the human, mono(2-ethylhexyl) phthalate (MEHP) was primarily produced in the liver followed by the intestine, kidney and lung. Mono(2-ethyl-5-OH-hexyl) (5-OH MEHP) and mono(2-ethyl-5-oxohexyl) (5-Oxo MEHP) phthalate were mainly produced by liver MIC (HLM) followed by intestinal MIC (HINT), while mono(2-ethyl-5-carboxypentyl) phthalate and mono(2-carboxy-methylhexyl) phthalate were found only in HLM. Metabolic activities for MEHP production in human were 19.39±0.9and 10.73±0.4 in HLM and liver Cytol, 17.79±0.2 and 8.02±1.2 in HINT and intestinal Cytol, respectively, 15.39±3.8 in kidney S9 (HKS9) and 5.95±1.7 in lung S9 (HLUS9) (nmol mg protein-1min-1). The metabolic activities for 5-OH MEHP and 5-Oxo MEHP were 47.65±0.7 and 0.46±0.01 in HLM and 0.15±0.001 and 0.03±0.003 (nmol mg protein-1min-1) in HINT, respectively. Phthalic acid (PA) in human was mainly produced in lung followed by brain, kidney, skin, intestine, testis and liver. In human the metabolic rate for PA was 80.18±0.91 in HLUS9, 68.59±18.51 in brain S9, 28.37±15.4 in HKS9, 3.88±0.8 in skin MIC, 3.09±0.4 in HINT and 2.25±0.9 (nmol mg protein-1min-1) in testis MIC, respectively. The metabolic rates for MEHP, 5-OH MEHP and 5-Oxo MEHP in rat liver MIC were 48.66±2.9, 14.55±0.6 and 0.05±0.001 (nmol mg protein-1min-1), respectively. Rat CYP2C6 (rCYP2C6) plays a major role in producing 5-OH MEHP followed by rCYP2C11, rCYP3A1 and rCYP3A2 while 5-Oxo MEHP was produced only by rCYP2C6. We previously reported that human CYP2C9*1 was mainly involved in the production of 5-OH MEHP and 5-Oxo MEHP. The metabolic activities for 5-OH MEHP and 5-Oxo MEHP were 323.37±67.82 and 3.71±0.61 by rCYP2C6 and 246.11±22.23 and 134.99±12.20 (pmol pmol CYP-1 min-1) by human CYP2C9*1, respectively. These data are being used in a PBPK model evaluation of cross-species dosimetry and human interindividual variability for DEHP exposures.

PS 1847 USE OF ALTERNATIVE PROMOTERS AS REGULATORS OF HUMAN MICROSOMAL EPOXIDE HYDROLASE EXPRESSION.

H. Nguyen, X. Yang and C. J. Omiecinski. *Center Molecular Toxicology, Penn State University, University Park, PA.*

Microsomal epoxide hydrolase (mEH; EHPHX1) is an important xenobiotic-metabolizing enzyme, involved in both detoxification and bioactivation processes of many reactive epoxide intermediates including as styrene 7,8-oxide, butadiene oxide, and epoxides generated by the monooxygenation of polyaromatic hydrocarbons. Previous results from our laboratory suggested that EPHX1 activities were highly correlated with EPHX1 protein levels but not with the corresponding mRNA levels. We therefore hypothesized that post-transcriptional regulation may participate in controlling EPHX1 activity in human tissues. In this study we used a 'rolling circle amplification - rapid amplification of cDNA ends' method to identify a new EPHX1 mRNA transcript variant, termed E1-b'. The E1-b' transcript is generated from a far upstream alternative promoter region, localized immediately downstream from the previously characterized E1-b alternative promoter. Of interest, the E1-b' transcript contains two upstream AUGs in its 5' GC-rich non-coding leader sequence, predicted to code for short peptide sequences of 26 and 17 amino acids in length, respectively. Using an in vitro transcription/translation system and transfection expression in mammalian cell lines we demonstrated that, compared to the E1 and E1-b variants, the presence of the E1-b' 5'-UTR significantly inhibited translation activity. Results from mutation analysis demonstrated that the two upstream open reading frames (ORFs) act synergistically to inhibit translation from the major ORF. Using a reporter assay system where the different EPHX1 5'-UTRs were fused to a luciferase gene, we confirmed the effect of E1-b' 5'-UTR on translation in transfected human cells in culture. Further, addition of the respective synthetic peptides to programmed reticulocyte lysates resulted in similarly reduced translational efficiencies of several mRNA transcripts. These results suggest that the presence of uORFs in the E1-b' transcript may function to modulate overall EPHX1 levels in human tissues.

PS 1848 RESPECTIVE ROLES OF CYP2A5 AND CYP2F2 IN THE BIOACTIVATION OF 3-METHYLINDOLE IN MOUSE OLFACTORY MUCOSA AND LUNG.

X. Zhou[1], J. D'Agostino[1], L. Li[1], G. S. Yost[2] and X. Ding[1]. [1]*Wadsworth Center, Albany, NY and* [2]*University of Utah, Salt Lake City, UT.*

3-Methylindole (3MI), a highly selective respiratory-track toxicant, can be metabolized by P450 enzymes to produce two potentially toxic metabolites, through distinct pathways: 3-methyleneindole (MEI), via dehydrogenation, and 2,3-epoxy-3MI, via epoxidation. The toxicity of 3-MI in the mouse lung and olfactory mucosa (OM) is believed to be related to the abundant, and tissue-selective expression of certain P450s enzymes, such as CYP2A5 and CYP2F2; the human orthologs, CYP2A13 and CYP2F1, are both very efficient in the bioactivation of 3MI. However, the role of CYP2A or CYP2F in 3MI bioactivation in lung and OM microsomal reactions has not been established, given the lack of a highly specific chemical inhibitor or antibody. The goal of the current study is to identify the roles of CYP2A5 and CYP2F2 in the bioactivation of 3-MI, by using recently generated Cyp2a5-null and Cyp2f2-null mouse models. The metabolites of 3MI by mouse OM and lung microsomes were identified in a NADPH- and glutathione (GSH)-fortified incubation. In incubations of 3MI with lung microsomes, two GSH adducts, 3-glutathionyl-S-methylindole (GS-A1) and 3-methyl-2-glutathionyl-S-indole (GS-A2), were found to be major products, with indole-3-carbinol (I-3-C) and 3-methyloxindole (MOI) also detected, as minor metabolites. In incubations of 3MI with OM microsomes, GS-A1, GS-A2, I-3-C, and MOI were all detected in ample amounts. Interestingly, in the Cyp2f2-null mice, the lung formation of GS-A1 and GS-A2 was significantly decreased, whereas the OM formation of all the metabolites, was not changed, compared to the WT mice. In contrast, in the Cyp2a5-null mouse, the lung formation of 3MI metabolites was not altered; but the OM formation of I-3-C and MOI were substantially decreased, compared with the WT mice. These findings indicate that while CYP2F2 plays a critical role in the bioactivation of 3MI in the lung, CYP2A5 plays an essential role in the bioactivation of 3MI in the OM. (Supported in part by NIH grant ES007462)

PS 1849 MICROSOMAL METABOLISM OF NAPHTHALENE (NA) IN TARGET AND NON-TARGET RODENT TISSUES: COMPARISON WITH NON-HUMAN PRIMATES.

A. Buckpitt, D. Morin, P. Edwards and L. Van Winkle. *Veterinary Medicine, University of California Davis, Davis, CA.*

NA is a volatile aromatic hydrocarbon to which humans are exposed. In rodents, NA causes dose dependent, species and tissue selective toxicity in murine airways and nasal epithelium, and rat nasal, but not airway, epithelium. Sites which are susceptible to acute toxicity are also targeted following long term exposure. Cytochrome P450 metabolism of NA plays a key role in the downstream tissue responses. The metabolism of NA has been characterized in a number of systems (whole tissue microsomes, postmitochondrial supernatant and airway explants) but side by side comparisons using microsomes prepared from only target areas of the respiratory tract have not been reported. Accordingly, these studies compared rates of microsomal NA metabolism in target areas of the respiratory tract of the mouse, rat and monkey. The highest rates of substrate turnover were in the rat nasal olfactory epithelium (30 nmoles/mg/min). Rates of metabolism in mouse olfactory microsomes (16.4) were half those in the rat. Microsomes from monkey nasoturbinates were less than 10% those of the mouse. Microsomes from dissected murine airways catalyzed NA metabolism at 10.9 nmole/mg/min whereas metabolism in rat airways occurred at 5% of this rate. The majority of the metabolites were accounted for as GSH conjugates of the 1,2-epoxide. At longer incubation times diGSH conjugates of the diepoxide and GSH adducts of the diol epoxide and 1,4-naphthoquinone were observed in all preparations except rat airway. Under the conditions used, less than 12% of the total metabolites produced were accounted for by 1-naphthol or dihydrodiol. Microsomes from mouse airways, mouse and rat nasal olfactory epithelium showed a high degree of stereoselectivity in NA epoxidation (20:1), rat airways and monkey nasal samples did not. Tissue susceptibility to NA-induced injury correlates with high rates of substrate turnover; metabolism in the nasal epithelium of monkeys is 10-50 fold lower than in rat olfactory epithelium. Supported by the Naphthalene Coalition under the API and by NCRR 00169.

PS 1850 CYTOCHROME P450S MEDIATE THE METABOLISM AND ARE REGULATED BY BERBERINE IN MICE AND HUMANS.

Y. J. Guo[1], F. Li[2], Y. Chen[1], Z. Tan[1], C. Pope[3], X. Chen[2], X. Ma[2], C. D. Klaassen[2] and H. Zhou[1]. [1]*Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Central South University, Changsha, China,* [2]*University of Kansas Medical Center, Kansas, KS and* [3]*XenoTech, LLC, Kansas City, KS.*

Berberine (BBR) is used in traditional medicine for many metabolic diseases. However, little is known about BBR metabolism and whether it influences the cytochrome P450 (CYP) enzymes in vivo. The purpose of this study was to characterize the metabolism of BBR in liver and its role in regulating cytochrome P450s. Metabolomic analysis identified 11 metabolites of BBR in urine and feces of mice, including 5 phase-I metabolites, which were predominant in feces, and 6 phase-II glucuronide and sulfate metabolites, which were predominant in urine. Of the 5 phase-I metabolites observed in mice, only 3 were detected when BBR was incubated with human and mice liver microsomes or recombinant human CYPs. CYP2D6 appeared to be the major enzyme producing the phase-I metabolites, followed by CYP1A2, 3A4, 2E1 and 2C19. BBR (10, 30, 100, 300 mg/kg, p.o.) was given to 8-week-old C57BL/6 male mice for 2 weeks. In the highest dosed group, Cyp3a mRNAs decreased about 75%, and the enzyme activity of Cyp3a and 2d decreased 83% and 67%, respectively. A two-phase randomized crossover clinical study in healthy male subjects showed that after 2-weeks of BBR, 300 mg t.i.d. p.o, the Cmax and AUC of midazolam (probe for CYP3A4) increased 36.5% and 45.0%, the oral clearance decreased 24.2%, the t1/2 prolonged from 3.03 h to 3.66 h, and the 1 hr ratio of midazolam/1'-hydroxymidazolam increased 49.6%. BBR also increased the 0-8 h urinary ratio of dextromethorphan/dextrorphan (CYP2D6 activity marker) by 900% and Losartan/E-3174 (CYP2C9 activity marker) by 100%. In conclusion, the metabolites of BBR are similar in human and mice. BBR treatment decreased CYP450 activities in mice and humans. Therefore, drug-drug interactions should be considered especially when high doses of BBR are taken (supported by NIH grants ES-09649, ES-09716, ES-07079, DK 081461 and RR-021940).

PS 1851 CYTOCHROME P450 2S1 MEDIATED REGULATION OF HUMAN LUNG CELL PROLIFERATION.

A. M. Rowland, T. W. Madanayake, T. P. Fidler and L. Montoya. *Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM.*

Cytochrome P450 2S1 (CYP2S1) is a drug metabolism enzyme that is expressed in hyperproliferative disease and metabolizes endogenous and exogenous chemicals linked to lung carcinogenesis and treatment. CYP2S1 is preferentially expressed in extrahepatic tissues and is elevated in hyperproliferative diseases including psoriasis and epithelial cancers. Recent studies demonstrate that CYP2S1 metabolizes Benzo[a]pyrene (B[a]P) to the ultimate carcinogen, B[a]P-diolepoxide. Under hypoxic conditions, CYP2S1 demonstrates a robust reductive metabolism of the prodrug AQ4N [1,4-bis{[2-(dimethylamino-N-oxide)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione] to the active topoisomerase II inhibitor, AQ4 [1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dihydroxy-anthracene-9,10-dione]. Conflicting metabolic results have been demonstrated for a potential endogenous substrate, all-trans retinoic acid (atRA). Using independent heterologous systems, CYP2S1 either metabolized or failed to metabolize atRA to the 4-hydroxy metabolite. To determine whether CYP2S1 plays a functional role in pulmonary cells, we examined the impact of changes in CYP2S1 expression on lung cell proliferation. We performed stable shRNA knock down of CYP2S1 expression in both human lung alveolar (A549) and bronchiolar epithelial cells (BEAS-2B). CYP2S1 mRNA levels were significantly reduced by approximately 70% in both A549 and BEAS-2B cells. CYP2S1 protein was reduced by 50% and 80% in A549 and BEAS-2B, respectively. Bronchial cells with reduced CYP2S1 expression exhibit elevated proliferation rates, as demonstrated through both in vitro proliferation assays and flow cytometry. These studies suggest an important physiological role for CYP2S1-mediated metabolism of unknown endogenous morphogen or mophogens.

PS 1852 NEW ROLES OF CYTOCHROME P4501A1 (CYP1A1) AND THE ENDOGEONUS ARYL HYDROCARBON RECEPTOR (AHR) LIGAND 6-FORMYLINDOLO[3, 2-b]CARBAZOLE (FICZ) IN REGULATION OF CELL GROWTH.

A. Rannug[1], H. Mei[1], A. Mohammmadi-Bardbori[1], Y. Wei[2] and U. Rannug[3]. [1]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden,* [2]*Department of Community Medicine, Mercer University School of Medicine, Macon, GA and* [3]*Department of Genetics, Microbiology and Toxicology, Stockholm University, Stockholm, Sweden.*

CYP1A1 is associated with the metabolism of xenobiotics. Our attention, however, has focused on the role of this enzyme in cell proliferation. CYP1A1 causes rapid turnover of FICZ, the candidate endogenous AHR ligand. Since the AHR has been implicated in the regulation of cell growth, we hypothesize that FICZ can stimulate cell cycle progression and that this function is controlled by CYP1A1. A pronounced increase in proliferative ability in cells grown at relatively high density in medium containing 1 μM FICZ was demonstrated, by using different experimental methods to document cell proliferation, with both human HaCaT cells and mouse Hepa-1 cells. Grown under such differentiated conditions, the cells express high CYP1A1 activity. To study the effects of FICZ under conditions of low CYP1A1 enzyme activity, HaCaT cells were grown undifferentiated at a low cell density. Under these conditions, cell growth was stimulated by FICZ, and DNA synthesis was enhanced as measured by FACS analysis, at concentrations as low as 1 pM. Interestingly, in Hepa-1 cells, we observed that CYP1A1-deficient c37 cells grow more rapidly than wild type Hepa-1 cells and that transfection with a plasmid containing a functional CYP1A1 cDNA inhibits the more rapid growth of c37 cells. In medium containing purified L-tryptophan (recrystallized to avoid background contamination with FICZ) the growth rate of c37 cells was not higher compared to wild type cells but addition of FICZ could stimulate proliferation of c37 cells at a concentration of 100 fM. Taken together, we propose that FICZ has vitamin/hormone-like physiological properties and that the AHR and CYP1A1 participate in an autoregulatory feedback that maintains the steady-state concentrations of FICZ at low levels in order to maintain homeostasis.

PS 1853 TRANSCRIPTOMIC EVALUATION OF CANINE SUSPENSION-SHIPPED AND PRE-PLATED HEPATOCYTES: COMPARISON TO LIVER.

A. Ditewig, M. Liguori, E. Blomme and Y. Yang. *Cellular, Molecular and Exploratory Toxicology, Abbott Laboratories, Abbott Park, IL.*

The dog is frequently used for preclinical toxicity testing in the pharmaceutical industry. In vitro tests using rat and human hepatocytes are commonly conducted; however these methods are far less established for dog hepatocytes. In particular, little is known about the effects of plating and culturing on hepatocellular functions. Our objective was to evaluate transcriptomic changes between hepatocytes and liver using gene expression profiling. Canine liver samples and hepatocytes isolated from these livers were shipped either in suspension or pre-plated (P). Upon arrival, an aliquot of each cell preparation was harvested in Qiazol and the remaining suspension-shipped (S) cells were plated at a density comparable to the P cells. Hepatocytes were cultured for 120 hours or were treated with 1 mM Phenobarbital (PB) and 20 μM Rifampin for 48 hours; RNA was isolated for RT-PCR and mi-

crorarray analysis. RT-PCR results indicated that basal levels of CYP3A12 and CYP2B11, two major metabolizing enzymes in the dog, were highest in the liver and declined over time in cultured hepatocytes. CYP3A12 levels declined more rapidly in S cells than in P cells with a more drastic difference occurring between the cell preparations after 72 hours. CYP3A12 induction by PB in both cell preparations was low but comparable despite the lower baseline of the S cells. A transcriptomic comparison between liver and cell preparations indicated that the transcriptome was significantly affected in cultures after plating. S hepatocytes harvested immediately upon receipt were most similar to liver; six hours after plating, the transcriptome of S cells began to change. A cluster analysis indicated that samples clustered according to harvest time point, not cell preparation. Genes regulating processes involved in phase I/II metabolism, transporter systems, lipid metabolism and oxidative stress were impacted by plating. These data allow for better interpretation of compound-related changes and limitations of the model in canine hepatocytes.

PS 1854 RAT CYTOCHROME P450 2S1 (CYP2S1) IS NOT INDUCED BY THE ARYL HYDROCARBON RECEPTOR (AHR) AGONIST PCB126.

B. Wang[1], L. Robertson[1, 2], K. Wang[3] and G. Ludewig[1, 2]. [1]*Human Toxicology, University of Iowa, Iowa City, IA,* [2]*Occupational & Environmental Health, University of Iowa, Iowa City, IA and* [3]*Biostatistics, University of Iowa, Iowa City, IA.*

CYP2S1 is an evolutionarily conserved member of the diverse CYP2 family. In mammals CYP2S1 is mainly expressed in extra-hepatic tissues. Since CYP2S1 mRNA and immunoreactive protein were shown to be increased by TCDD in several tissues in rodents, it was proposed that CYP2S1 is regulated by the AhR pathway. The present study was aimed at further exploring the possible regulation of CYP2S1 through the AhR using PCB126, the most potent AhR agonist among all PCBs . In a time-response study male Sprague Dawley rats received a single i.p. injection of 5 μmol/kg PCB126 and were sacrificed at different time points after exposure. CYP1A1, CYP1A2, CYP1B1 and CYP2S1 relative mRNA expression was determined in liver, lung, spleen, stomach, kidney, and thymus tissues using qRT-PCR. In addition, the expression of CYP2S1 was examined after treatment of rats with various classical CYP-inducers that act through different receptors, i.e. β-naphthoflavone (β-NF, an AhR agonist), phenobarbital (PB, a CAR activator) and dexamethasone (Dex, a PXR activator); here CYP2B, CYP3A1, and above CYPs were measured in liver and lung. Our results show that in untreated rats CYP2S1 was expressed at comparable levels to other CYPs in extra-hepatic organs with the highest levels detected in stomach, kidney and lung. PCB126 did not increase CYP2S1 mRNA levels in any organ and at any time point examined despite of a significant induction of CYP1 genes. Conversely, CYP2S1 mRNA was reduced by 40% starting from the 7th day post exposure in thymus. With β-NF the CYP2S1 mRNA level was slightly, but not significantly elevated in liver tissues. PB and Dex had no effect on CYP2S1 levels. All 3 inducers produced the expected effects on the other CYP enzymes. Collectively, these observations suggest that CYP2S1 is not or only weakly regulated by the AhR and not at all by CAR or PXR in SD rats. (Supported by NIEHS P42 ES013661 and DAMD17-02-1-0241)

PS 1855 REGULATION OF CARBONYL REDUCTASE EXPRESSION AND ACTIVITY BY AH RECEPTOR LIGANDS.

E. A. Amouzougan[1], C. R. Klocke[1], H. A. Charlier[2] and K. A. Mitchell[1]. [1]*Biological Sciences, Boise State University, Boise, ID and* [2]*Chemistry and Biochemistry, Boise State University, Boise, ID.*

Carbonyl reductase (CBR) is a NADPH-dependent enzyme that reduces various carbonyl-containing compounds, including the anthracycline family of chemotherapy agents. CBR reduces anthracyclines to their alcohol metabolites, which have reduced cytotoxic activity compared to the parent compound and also display cardiotoxicity. Hence, inhibiting CBR expression and/or activity is a potential strategy to increase the efficacy and decrease the toxicity associated with anthracycline chemotherapy. Recent evidence links aryl hydrocarbon receptor (AhR) signaling to the expression of CBR protein. The goal of the present study was to investigate how exposure to AhR agonists and antagonists modulate CBR expression and activity, and to determine how such changes impact the efficacy of the anthracycline antibiotic, doxorubicin. We found that doxorubicin regulates CBR expression by an AhR-dependent mechanism, as it dose-dependently induced CBR expression in 5L rat hepatoma cells, but not in the AhR-deficient hepatoma cell line, BP8. Treatment of 5L cells with the AhR agonist beta-napthoflavone (bNF) also dose-dependently increased CBR expression. However, this increase was not sufficient to modulate the IC_{50} for doxorubicin in 5L cells (200 nM). However, treatment of 5L cells with AhR antagonists resveratrol or triclosan significantly enhanced doxorubicin-mediated cytotoxicity. This effect may be due to suppression of CBR expression in doxorubicin-treated cells. However, it could also result from a direct effect on the CBR enzyme, as both of these AhR ligands suppressed activity of the native CBR enzyme in a cell-free preparation. Hence, AhR antagonists may provide a novel approach to enhancing the efficacy of anthracycline chemotherapy by inhibiting CBR expression and/or activity.

PS 1856 INCREASED LEVELS OF OXIDATIVE DNA ADDUCTS IN CYTOCHROME P450 (CYP)1A2-NULL MICE AND PREMATURE INFANTS EXPOSED TO HYPEROXIA: NOVEL BIOMARKERS FOR BRONCHOPULMONARY DYSPLASIA (BPD).

B. Moorthy[1], L. Wang[1], C. Xanthi[1], G. Zhou[2] and W. Jiang[1]. [1]*Pediatrics, Baylor College of Medicine, Houston, TX and* [2]*Institute of Biotechnology, Texas A&M University, Houston, TX.*

Supplemental oxygen therapy is frequently encountered in the treatment of infants having pulmonary insufficiency. However, prolonged exposure to hyperoxia contributes to BPD in infants. In this investigation, we tested the hypothesis that mice lacking the gene for CYP1A2 would display increased susceptibility to oxidative DNA damage in vivo in response to hyperoxia, compared to wild type (WT) mice. We also tested the hypothesis that tracheal aspirates of premature infants who develop BPD will display higher levels of oxidative DNA adducts. Twelve week-old male WT (C57BL/6J) mice or Cyp1a2-null mice were exposed to hyperoxia (greater than 95% O2) or room air for 24-72 h. In another experiment, tracheal aspirates from premature infants who received supplemental oxygen (6 BPD group and 5 non-BPD group) were used to measure oxidative DNA adducts 5 days after birth. Oxidative DNA lesions in lung or liver tissues of mice or human tracheal aspirates were determined by nuclease P1-enhanced version of the 32P-postlabeling assay. The Cyp1a2-null mice were more susceptible to lung damage than similarly exposed WT animals, as determined by histology. Oxidative DNA adducts were augmented in the lungs of WT and Cyp1a2-null mice exposed to 72 h of hyperoxia, compared to room air controls, with Cyp1a2-null mice showing a greater degree of enhancement. In a pilot study using tracheal aspirates, the oxidative DNA lesions were significantly higher in the BPD group than the non-BPD group. The positive correlation between oxidative DNA adducts and extent of lung injury suggests that oxidative DNA damage contributes to lung injury. Our human data suggest that oxidative DNA adducts could be developed as novel biomarkers for BPD. Further studies could lead to the development of novel strategies for the prevention/treatment of BPD in infants.

PS 1857 ALTERATION IN EXPRESSION OF MOUSE CARBOXYLESTERASE ISOFORMS FOLLOWING DEVELOPMENTAL DELTAMETHRIN EXPOSURE: ROLE OF NUCLEAR RECEPTORS.

A. A. Baker and J. R. Richardson. *Toxicology, UMDNJ-Rutgers University, Piscataway, NJ.*

Previously we reported that developmental exposure to deltamethrin increased Phase I enzyme carboxylesterase (Ces) protein (139%) along with Ces enzyme activity (215%) in male offspring (Baker et al., 2009). Induction of Ces was associated with activation of the nuclear receptor-mediated pathways involving CAR, and possibly PXR. Here, we sought to determine the relative contribution of different Ces isoforms (Ces1(Es/x), Ces2(2a3), Ces3(TGH), Ces5(BC022148), Ces6, EsD(Es10), Es1(EsN or Es4), Es22(egasyn), Es31) to the increase in Ces protein and activity following developmental deltamethrin exposure. Pregnant C57BL/6j mice were administered 0 or 3 mg/kg deltamethrin every three days throughout gestation and lactation. Livers were collected from 11-12 month old male offspring for mRNA analysis. Developmental deltamethrin exposure significantly increased mRNA expression levels of Ces1(Es/x), Ces3(TGH) and Ces6 by 104%, 85%, 105%, respectively. To determine the role of nuclear receptor-mediated pathways in the induction of Ces isoforms, adult mice were treated with inducers of CAR (TCPOBOP) and PXR (PCN). CAR activation by TCPOBOP increased the mRNA expression of Ces1, 3, and 6 isoforms by 17%, 105% and 883%, respectively. PXR activation by PCN increased Ces1, 3, and 6 isoforms mRNA expression by 152%, 126% and 522%, respectively. These data demonstrate the ability of developmental deltamethrin exposure to persistently induce multiple isoforms of

mouse Ces, which could potentially affect the disposition and metabolism of pharmacological and toxicological agents. Moreover, these data demonstrate the involvement of nuclear receptor mediated regulation of Ces by CAR as well as PXR, and suggest possible crosstalk between the two, following developmental deltamethrin exposure. Supported by NIEHS R01ES015991, P30ES005022, and T32ES007148.

PS 1858 TRANSCRIPTIONAL REGULATION OF THE HUMAN MICROSOMAL EPOXIDE HYDROLASE GENE (EPHX1) DRIVEN BY A FAR UPSTREAM ALTERNATIVE PROMOTER.

S. Su[1, 2] and C. Omiecinski[2]. [1]*Huck Institute of Life Sciences, Penn State University University Park, PA and* [2]*Center for Molecular Toxicology and Carcinogenesis, Penn State University University Park, PA.*

Microsomal epoxide hydrolase (EPHX1) plays an important role in the detoxification of xenobiotic epoxide intermediates generated by CYP450s. However, it is also involved in the bioactivation of pro-carcinogens, such as polyaromatic hydrocarbons. The transcription of the human EPHX1 gene is initiated from alternative promoters; two alternative transcripts are denoted E1 and E1b, respectively. Our previous studies demonstrated that the E1-contained transcripts are expressed selectively in liver, whereas the E1b promoter predominantly drives the expression of EPHX1 in all human tissues. Although the levels of the E1b transcript are tissue-dependent, the regulatory mechanisms for tissue-specific control of E1b transcript expression remain unknown. To begin to elucidate these mechanisms, we determined that the E1b basal promoter activity is maintained within a promoter region of 300bp immediately proximal to the E1b transcriptional initiation site. In silico analysis of this region revealed the presence of several candidate binding motifs for SP1. In this study, we conducted site-directed mutagenesis of these SP1 elements, coupled with gene reporter assays to demonstrate important functional roles contributed by SP1 in the regulation of EPHX1 transcription. Further, E1b promoter activities were increased significantly following SP1 overexpression in human cell cultures. These results establish SP1 as a major regulator of E1b promoter activity in human tissues. Since methylation of CpG islands within gene promoter regions has been shown previously to transcriptionally repress genes regulated by SP1, we hypothesize a relationship between the methylation status of the CpG islands within the SP1 region of the E1b promoter and EPHX1 tissue specific expression. Current efforts are being directed to determine these relationships and to assess the contribution of E1b promoter methylation to the tissue-specific expression patterns of human EHPX1.

PS 1859 ROS PRODUCTION AND ENZYME INACTIVATION OF ZEBRAFISH CYP1S BY HALOGENATED AROMATIC HYDROCARBONS.

J. V. Goldstone[1], P. Urban[2], D. Pompon[2] and J. J. Stegeman[1]. [1]*Biology, Woods Hole Oceanographic Institution, Woods Hole, MA and* [2]*CNRS-Gif sur Yvette, Gif sur Yvette, France.*

Halogenated aromatic hydrocarbons (HAHs), including PCBs and polychlorinated dibenzo-p-dioxins elicit oxidative stress. HAH toxicity in zebrafish embryos can be prevented by antioxidants. Zebrafish have five cytochrome P450 (CYP1) enzymes, CYP1A, CYP1B1, CYP1C1, CYP1C2, and CYP1D1.and knock-down of CYP1A and CYP1Cs also has been suggested to protect against some phenotypes of HAH toxicity. Prior work has shown microsomal CYP1A to be irreversibly inactivated by planar PCBs and tetrachlorodibenzo-p-dioxin in a oxygen-, NADPH-, and time-dependent process, accompanied by the release of reactive oxygen species (ROS). Little is known about possible HAH substrates and the potential for oxidative uncoupling of the CYP1C and CYP1D enzymes. We have examined the quantitative relationship between NADPH consumption, ROS release, and enzyme inactivation, using heterologously expressed CYP1s. CYP1C1, CYP1C2, and CYP1D1 were expressed in W(R) yeast that overexpresses P450 reductase (POR). We fluorometrically quantified the production of hydrogen peroxide using Amplex Red, and spectrophotometrically determined the release of superoxide using acetylated cytochrome c. NADPH oxidation is followed spectrophotometrically to examine the kinetic rates of HAH-dependent enzyme inactivation and associated kinetic parameters of the HAH-NADPH-POR-CYP1 system. Uncoupling was measured as the HAH-stimulated production of significant amounts of superoxide and hydrogen peroxide by the expressed proteins. Rates of enzyme inactivation were decreased by the addition of increasing concentrations of DMSO. As DMSO is a small-molecule radical scavenger, suggest that hydroxyl radical generated in the active site could be responsible for the HAH-dependent enzyme inactivation. (NIH R01ES015912 and P42ES007381).

PS 1860 OXIDATIVE METABOLISM OF STYRENE IN CYP2F2-NULL MOUSE LIVER AND LUNG MICROSOMES.

S. Shen[1], L. Li[2], X. Ding[2] and J. Zheng[1, 3]. [1]*Center for Developmental Therapeutics, Seattle Children's Research Institute, Seattle, WA,* [2]*Wadsworth Center, New York State Department of Health, and School of Public Health, State University of New York, New York, NY and* [3]*Department of Pediatrics, University of Washington, Seattle, WA.*

Metabolic activation is considered to be a critical step for styrene-induced pulmonary toxicity. Styrene-7,8-oxide (SO) is a primary oxidative metabolite generated by vinyl epoxidation of styrene. Additionally, urinary 4-vinylphenol (4-VP), a phenolic metabolite formed by aromatic hydroxylation, has been detected in workers and experimental animals after exposure to styrene. Recently, we identified several new oxidative metabolites of styrene, including 2-vinylphenol (2-VP), 3-vinylphenol (3-VP), vinyl-1,4-hydroquinone, and 2-hydroxystyrene glycol, in mouse liver microsomal reactions. In the present study, we investigated the role of CYP2F2, which is expressed in both lung and liver, in the generation of the oxidative metabolites. An approximately 50% reduction was observed in the rates of the formation of SO (styrene glycol was monitored) in liver and lung microsomes prepared from Cyp2f2-null mice relative to the wild-type mice. Substantial decreases in the formation of VPs, vinyl-1,4-hydroquinone, and 2-hydroxystyrene glycol were also found in the Cyp2f2-null microsomal incubations with styrene. Metabolic oxidation of 2-, 3-, and 4-VPs was evaluated in Cyp2f2-null and wild-type mouse liver and lung microsomes as well. A tremendously decreased metabolism of the VPs to the corresponding catechols/hydroquinone and oxides (vinyl epoxides) was observed in Cyp2f2-null mouse lung microsomes, compared with that of the wild-type mice. These results indicate that CYP2F2 is involved in both primary and secondary metabolism/bioactivation of styrene.

PS 1861 DOWN REGULATION OF MOUSE CYP2DS IN PRIMARY HEPATOCYTES USING RNAI.

O. Elraghy[1], B. Damiri[2] and W. S. Baldwin[1, 2]. [1]*Biological Sciences, Clemson University, Clemson, SC and* [2]*Environmental Toxicology Program, Clemson University, Clemson, SC.*

The Cyp2d subfamily is the second most abundant subfamily of hepatic drug metabolizing CYPs. In mouse, there are nine mouse Cyp2d subfamily members that are believed to have redundant functions. In humans, CYP2D6 is the only Cyp2d member, but it is highly polymorphic with over 90 known allelic variants, resulting in a number of adverse drug reactions. We are using siRNA to knockdown the expression of multiple mouse Cyp2ds in primary mouse hepatocytes with one siRNA construct. Expression of only Cyp2d10, Cyp2d11, Cyp2d22, and Cyp2d26 were observed in the primary male mouse hepatocytes. Cyp2d9, which is male specific and growth hormone-dependent, was not expressed in male primary hepatocytes, potentially because of its growth hormone dependence. Several transfection reagents were used and it was found that for the best silencing efficiency coupled with low cell toxicity, Mirus TKO-transfection reagent specifically designed for primary cells worked best. Transfection reagents designed for primary cell culture showed greater transfection effiency than those designed for cell culture with immortal cell lines. Several different siRNAs at different concentrations were used to knockdown Cyp2d expression; some of which were designed to repress only one construct. siRnA constructs designed to repress only one construct often repressed several Cyp2d isoforms. A construct designed to knockdown every Cyp2d isoform, siRNA-KD1, provided the best results as it repressed the expression of every isoform. Interestingly, siRNA-KD2, a construct designed to knockdown all Cyp2d isoforms, except Cyp2d10 caused a 2.5-fold increase in Cyp2d10 expression, presumably because of a compensatory response. Overall, this study provides a new tool for determining Cyp2d's role in xenobiotic metabolism, valuable information on how to design siRNA studies for silencing multiple murine Cyps, and ultimately may help in silencing mouse Cyp2d's within the humanized CYP2D6 mouse model.

PS 1862 DIFFERENTIAL SULFOTRANSFERASE EXPRESSION AFTER FASTING AND CALORIC RESTRICTION IN LIVERS OF LEAN (C57BL/6) AND OBESE (OB/OB) MICE.

N. H. Knudsen[1], S. R. Kulkarni[2], J. Xu[2], R. S. King[2] and A. L. Slitt[2]. [1]*Biochemistry, Moravian College, Bethlehem, PA and* [2]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Bethlehem, RI.*

A large portion of Americans are currently undergoing caloric restriction (CR) or fasting in an effort to reverse obesity, type-II diabetes mellitus, and non-alcoholic fatty liver disease (NAFLD). Several biotransformation enzymes and transporters are differentially expressed in livers of obese (ob/ob) mice. Sulfotransferases (Sults)

are a family of phase-II biotransformation enzymes, which conjugate hormones, bile acids, drugs and environmental chemicals to form more water-soluble metabolites for excretion and aid in drug detoxification. Little published information exists regarding how either obesity and NAFLD, or nutrient deprivation affect Sult expression in liver. In the current study, mRNA expression of eight Sult isoforms (1a1, 1b1, 1d1, 1e1, 2a1, 2b1, 4a1 and 5a1) was determined by the Branched DNA Signal Amplification Assay. mRNA was isolated from livers of adult C57Bl/6 (lean) and ob/ob (obese) male mice that were 1) fed or fasted (24 or 30 hours), or 2) fed ad libitum (AL) or placed on CR (40% reduced caloric intake) for 10 weeks. Obesity increased Sult2a1 and 1e1 mRNA expression and decreased Sult5a1 expression as compared to lean controls. Twenty-four hour fasting increased Sult1a1 (3.2 fold), 1d1 (6.5 fold) and 1e1 (3.7 fold) expression compared to fed controls in lean mice only. Thirty hour fasting increased Sult1a1, 1d1 and 1e1 expression in livers of obese mice by 1.5, 1.5 and 2.3 fold, respectively, over fed controls. CR decreased Sult1b1 and 5a1 mRNA expression to 18% and 20%, respectively, in livers of lean mice and increased Sult1e1 mRNA expression by 2.3 fold in livers of obese mice, as compared to fed controls. Our data indicate that fasting and CR differentially affect Sult expression in livers of lean and obese mice. Future studies will address mechanisms by which Sults are regulated during obesity and whether Sult activity is altered in these models.

PS 1863 NAT1 INTERACTION WITH PPD AND OXIDIZED PPD.

J. Bonifas, D. Dierolf and B. Blömeke. *Environmental Toxicology, University Trier, Trier, Germany.*

N-acetyltransferase 1 (NAT1)-mediated N-acetylation is an important detoxification pathway for the hair dye ingredient and well-known contact allergen paraphenylenediamine (PPD). However, the N-acetylation capacity can be modulated by several mechanisms. We demonstrated recently that NAT1 activity in keratinocytes can be reduced by PPD and p-aminobenzoic acid (PABA) based on substrate-dependent NAT1 protein degradation. Furthermore, modifications of cysteine in the active centre of NAT1 by oxidation or covalent binding of reactive chemicals can also impair NAT1 activity. In the present study, we asked if PPD or oxidized PPD can directly influence NAT1 activity beyond the observed substrate dependent mechanism.
Recombinant human NAT1*4 (baculovirus expression system) was incubated for 6h and 16h with 1 mM PPD and oxidized PPD (air oxidation for 24h). Iodoacetamide (10 μM) and 2 mM hydrogen peroxide were used as positive control for direct NAT1 activity inhibition. Enzyme activities were determined by quantification of coenzyme A via staining with 5,5'-dithio-bis(2-nitrobenzoic acid) after N-acetylation (30 min) of 1 mM PABA by 2.5 or 1 ng/μl protein in the presence of 1 mM acetyl coenzyme A .
PPD treatment resulted in 70% inhibition of NAT1 activity after 16 h, whereas oxidized PPD reached only 41% NAT1 inhibition. A 6h treatment led to an average NAT1 inhibition of 20% and 17.5% by PPD and oxidized PPD, respectively. The activity of NAT2 was not reduced by the treatment. Preliminary experiments on reversibility of this inhibition showed that treatment of NAT1 with PPD or oxidized PPD in the presence of 1 mM GSH slightly reduced NAT1 inhibition, suggesting that the NAT1 inhibition is partly reversible by this reducing agent.
These data indicate that NAT1 inhibition by PPD was stronger compared to oxidized PPD. In conclusion, these results and our recently published data demonstrate that PPD can inhibit NAT1 activity directly as well as by substrate-dependent NAT1 degradation.

PS 1864 CHANGES IN PHASE I AND PHASE II ENZYME ACTIVITIES OVER TIME IN PLATED HUMAN HEPATOCYTES.

T. Moeller, S. Heyward, J. Lee and R. Tolando. *Celsis in vitro Technologies, Halethorpe, MD.* Sponsor: M. Xia.

It is well known that P450 activities in hepatocytes will diminish over time in cultured hepatocytes as documented in studies using rat cultured hepatocytes and human liver slices measuring P450 activities. Other reports have included P450 mRNA losses in human cultured hepatocytes but without comparative enzyme activities of the major drug metabolizing CYP enzymes. Here, we report measuring the P450 enzyme activities along with UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) in cultured human hepatocytes. Plateable cryopreserved human hepatocytes were used to determine the enzymatic activity of CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4, as well as UGT and SULT over a time course of 36 hours. There was an increase in activities for CYP2B6, 2C9, 2C19 and 2E1 in the first four hours after attachment, while the other CYPs decreased. All CYP activities trended down at the 12-, 18-, 24- and 36-hour time points. The raw activities did vary from donor to donor; however, the trend in the percent loss was similar in most CYPs with average T1/2 of 22.9 hours. UGT activity increased over first four hours, then remained stable out to 36 hours. SULT activity varied from donor to donor with an increase in the first four hours and then differences in maintaining the activity to 36 hours. One donor loss activity from the four hour value but maintained approximately the initial value measured. The other donors retained their initial increases in activity. In all, we confirmed the reported loss of activities in human hepatocytes and the stability of UGT activity. Retention of SULT activity was unique to the reported loss in human liver slices and rat hepatocyte cultures.

PS 1865 INSPECTOR TOXICOLOGY : AN INNOVATIVE TOXICOLOGY AWARENESS PROGRAM.

D. Hardej[1, 2] and A. R. Schatz[1]. [1]*Mid-Atlantic Society of Toxicology (MASOT), Bordentown, NJ and* [2]*Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY.*

Inspector Tox is an educational program that was developed by the MASOT Education and Outreach Committee to raise awareness of toxicology in young audiences. Inspector Tox is a Sherlock Holmes-like character who initially introduces the audience to basic concepts of toxicology and then leads into "toxic scenes" that take place in the home, in the yard and at the beach. The scenes were designed to incorporate a variety of xenobiotics, including toxins, industrial toxicants, as well as behavioral /recreational toxicants such as cigarette smoke, drugs of abuse and alcohol. The audience is given an investigators notebook in which they fill in questions presented in the introduction as they interact with Inspector Tox. As the scenes progress, the audience is asked to distinguish between potentially harmful and safe activities. As each scene is completed, Inspector Tox interacts with the audience to solicit and discuss responses. The program was developed initially by the Chair of the MASOT Education and Outreach Committee. Modification of dialog for the introduction and the toxic scenes was accomplished by interaction with the Committee members who represent academia, industry and government experts in toxicology to ensure that relevant and current topics were covered. The audience for the program resulted from a joint effort of St. John's University and the Afterschool All Stars Program. The Afterschool All Stars is a program that provides free programs to close to 80,000 children in need in 13 different cities in the continental U.S. and Hawaii. Volunteers were recruited from MASOT, St. John's faculty, students and administrators. Learning outcomes for the program are currently based on responses solicited from the audience and written in the investigator notebook provided. Adaptation for younger audiences (grades 1-3) were made using booklets that provided pictures to circle rather than written responses to the toxic scenes.

PS 1866 TOTALLY TOXIC! A K-12 SCIENCE OUTREACH ACTIVITY USING CASE STUDIES TO TEACH BASIC PRINCIPLES OF TOXICOLOGY.

C. P. Curran[1] and D. R. Mattie[2]. [1]*Biological Sciences, Northern Kentucky University, Highland Heights, KY and* [2]*Nano/Toxicology Section, AFRL/Wright-Patterson AFB, Dayton, OH.*

The Ohio Valley Society of Toxicology (OVSOT) has established the position of K-12 liaison to encourage toxicology education in area schools. A program, "Totally Toxic!" was developed and tested at schools participating in the Greater Cincinnati and Northern Kentucky Girls in Science programs. The target audience was middle school girls, because research indicates this is a critical age for girls deciding whether to continue taking science and math courses. Totally Toxic! uses current and historic case studies to demonstrate essential principles of toxicology such as "The dose makes the poison." In addition, students are trained to identify toxins and toxicants in common household products. They learn that individual factors such as age, genetics, and sex can affect their response to toxicant exposure. Other important lessons include differential toxicities based on the route of exposure and co-exposures. The activity ends with Tox Teams solving "The Strange Case of Jennifer Strange," whose 2007 death was attributed to water intoxication. This activity serves as the assessment of the student learning outcomes, allowing for further elaboration and clarification if required.

PS 1867 SCIENCE DAY: HANDS-ON SCIENTIFIC OUTREACH WITH THE GIRL SCOUTS.

T. R. Hiltke[2] and M. R. Gwinn[1]. [1]*U.S. EPA, Washington, DC and* [2]*Girl Scout Council National Chapter, New Market, MD.*

Scientific experiments in elementary classrooms have been limited in recent years 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 of study. In an effort to increase enthusiasm for science, the Girl Scouts offer multiple badges in scientific education, but can only

supply limited guidance for specific experiments. Since 2007, the Girl Scout Council Nation's Chapter (GSCNC) Service Unit 37-7 (Oak Ridge Crossing) has designed hands-on activities for elementary students in different areas of science, including toxicology, as part of an annual Science Day. For the past two years, this event has been co-sponsored by National SOT and NCAC SOT through the Education Committee. This scientific outreach effort directly addresses the local (NCAC) and National SOT missions to encourage and support K-12 science education. Science Day is divided into two half-day sessions divided by age group and themed activities. Girl Scouts register for their age group and one of two themed sessions based on Girl Scout patches (e.g., Science Discovery or Science Sleuth). Each patch is earned after students complete the four to five activities in that session. Each activity is designed for a 30-minute time period, during which older Girl Scouts (grades 6 – 11) lead the lesson for 12 – 15 younger Girl Scouts. Since the first Science Day in 2007, Girl Scouts and SOT have reached out to more than 300 students per year, with numbers increasing every year. The additional funding and involvement of SOT scientists in the past two years has contributed to this increase, and to the complexities of the experiments provided. Science Day has served as pilot for various other hands-on activities in toxicology, with lessons learned from this activity each year helping to create greater interest in science for elementary school children, and to streamline similar events throughout the region. DISCLAIMER: This abstract does not represent EPA policy.

PS 1868 SCIENTIFIC OUTREACH—COLLABORATING WITH LOCAL PARTNERS.

M. K. Stapleton[2], M. R. Gwinn[1] and J. M. Matheson[3]. *[1]U.S. EPA, Washington, DC, [2]Towson University, Baltimore, MD and [3]Consumer Product Safety Commission, Bethesda, MD.*

Scientific literacy of kindergarten – 12th grade (K-12) students is a nationwide concern and K-12 schools cannot address this growing problem in a vacuum. Partnerships among K-12 schools, institutions of higher education and the scientific community are critical to addressing this problem. In 2009, the Society of Toxicology (SOT) and Towson University's Bioscience Education and Outreach (TU BEOP) program collaborated to provide a meaningful K-12 outreach effort at the SOT annual meeting in Baltimore, MD. This partnership leveraged both the informal science education experience already present in the local community (through TU BEOP) and the scientific expertise of SOT members (both local and national). This model outreach effort involved high school students, assisted by SOT volunteers, delivering hands-on, toxicology based activities to primary school students. The high school students underwent training by TU BEOP staff to provide them with the necessary scientific content knowledge to deliver the variety of short, hands-on toxicology-based science activities to local elementary school students. In addition to teaching younger students about toxicology-based science, the high school students were provided with an opportunity to conduct their own toxicology-based science experiment. Further, twelve high school students were invited to present their individual research in a poster session as part of the Annual meeting. As a result of this outreach partnership, 28 high school students and 40 SOT volunteers were able to present toxicology based science activities to over 700 individuals, including kindergarten and elementary students and their families. We suggest this outreach model can be used successfully by many professional societies and associations to increase awareness of, understanding and interest in their scientific work in the local communities where their annual meetings are held. This abstract reflects the views of the authors and does not necessarily represent the policies of their respective institutions.

PS 1869 THE IDAHO SCIENCE & ENGINEERING FESTIVAL: SATELLITE EVENT FOR K-12 SCIENCE EDUCATION.

J. C. Pfau, C. Evilia and L. DeVeaux. *Biological Sciences, Idaho State University, Pocatello, ID.*

The USA Science & Engineering Festival aims to re-ignite young people's interest in science, technology, engineering and math (STEM) through an exciting, hands-on and creative experience. The national event will culminate with a two day Expo on the National Mall, October 23/24, 2010, celebrating science and engineering in general but with special programs in clean technology, alternative energy, green chemistry, bio-mimicry, sustainability, ecology and conservation. The Festival's participants include leaders in technology from education, industry and government. Our goal was to create a local concurrent satellite event showcasing science in Idaho. First, we asked Idaho State University (ISU) to be a sponsor and host, helping with graphics, logistics, venue, budgeting, and account management. Second, potential sponsors across Idaho were contacted with a carefully crafted document that used the National Festival's excellent promotional rationale to explain what we were trying to do. Response was excellent, with sponsors ranging from small "door prize" donations to thousands of dollars. Third, we asked local scientists and engineers to provide booths, each with hands-on activities and fun learning opportunities for K-12 students. We have 30 of these activities, ranging from sponsor booths to presentations by faculty and academic clubs at ISU. Fourth, we contacted local schools, through school district administrators, for permission to distribute fliers and visit schools during science classes or assemblies to invite them to participate. To qualify for prizes, students will have a "passport" that must be stamped at several booths, encouraging them to engage in many activities at the Festival. We hope to increase community engagement in science and technology and create a greater awareness of science by staging a robust and inventive festival in Idaho; to attract enthusiastic participants; to attract a large audience of all ages, and to raise funds to support the Festival for years to come. Data will be collected to allow us to track the success of the Festival.

PS 1870 THE VOYAGE OF THE ODYSSEY: A UNIQUE PLATFORM FOR TOXICOLOGY RESEARCH AND EDUCATION.

I. Kerr[1, 3] and J. P. Wise[1, 2, 3]. *[1]Ocean Alliance, Lincoln, MA, [2]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME and [3]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME.*

The research vessel Odyssey is a 93-foot ketch motorsailer. It is equipped with state-of-the-art acoustic and sampling equipment for tracking and biopsying marine mammals, particularly whales. She is also equipped with state-of-the-art equipment for toxicological studies including cell culture and environmental sampling (water, air and prey). Odyssey sleeps 12 people and can stay offshore for weeks at a time. Thus, this vessel is a unique research platform for studying the toxicological impacts of environmental pollutants on whales and other marine mammals. However, Odyssey also has an important educational mission, engaging a broad spectrum of students in studying and understanding marine toxicology. For example, on her most recent voyage investigating the Gulf of Mexico oil crisis, 15 students worked at sea. Many more were engaged through working on field samples in the land laboratory or through daily logs sent out from the boat. Some students took online courses and stayed on board for the entire voyage while others rotated in for a leg of 1-2 weeks. Students were responsible for managing and performing all scientific aspects of the expedition as well as learning to work and sail the boat and raise funds for the expedition. One undergraduate, a rising freshman, setup the cell culture laboratory (the only one of its kind at sea) and became the first person in history to culture whale cells at sea and the first to culture fin whale cells. One undergraduate was a primary biopsier and collected more than 50% of the biopsies taken and ran the air-sampling program. Another undergraduate ran the water and sediment sampling program. All gained firsthand experience in working at sea, and in understanding the impacts and importance of toxicology in general and marine toxicology in particular, making Odyssey's educational and scientific missions a significant success.

PS 1871 REINFORCING QUANTITATIVE SKILLS THROUGH DATA ANALYSIS IN AN UNDERGRADUATE TOXICOLOGY COURSE.

V. A. Fitsanakis. *Biology, King College, Bristol, TN.*

In many college science and math courses, students are presented with and analyze idealized laboratory data. While greatly simplifying analysis, this often results in confusion and anxiety when students are later presented with real-world results. In order to reinforce quantitative skills (graphing, data and statistical analyses, and interpretation) and prepare students for biomedical research, students (n = 16) were presented with original neurotoxicology data in four successive labs. They were instructed to appropriately graph the data (bar graph versus line graph), determine whether to use standard deviation (SD) or standard error of the mean (SEM), and assess whether the data could be extrapolated to human populations. Prior to the labs, students anonymously answered pre-lab questions related to their data analysis skills. During the three-hour lab, students worked together in small groups discussing, analyzing and graphing the data. After completing each assignment, students anonymously answered the same questions presented in the pre-lab assessment to determine whether the lab had reinforced various mathematical and analytical skills. For each session, the majority students (60-80%) reported "Strongly Agree" or "Agree" when asked if they were confident in their ability to successfully analyze the data. This was in stark contrast to their responses prior to the lab, where the majority (60-80%) reported that they would "Disagree" or "Strongly Disagree" with the same statements. Their confidence was also noted in the change in SD from the first lab (average grade 83.11 + 11.39) to the lab exam (85.67 + 4.92). Although the average score was statistically the same, the SD was much small, suggesting that more students were closer to the average that in previ-

ous labs. As a result of positive student comments and the increase in their understanding of data analysis and graphing, this series of assignments will continue to be used in future Mammalian Toxicology (BIOL 4670) classes.

PS 1872 UNDERGRADUATE RESEARCH UTILIZING *C. ELEGANS* DURING A TOXICOLOGY COURSE.

M. Pomeroy-Black. *LaGrange College, LaGrange, GA.*

Encouraging undergraduate science majors to pursue graduate school in the field of toxicology can be a challenge. A toxicological inquiry experiment provides an opportunity for students to learn about scientific research methods and engage in a graduate school experience. Because the soil nematode Caenorhabditis elegans is easily maintained, is hardy and requires little instrumentation, it naturally lends itself to research with undergraduate students. During one semester, pairs of undergraduate students in a Toxicology course conducted an inquiry experiment utilizing C. elegans. They were provided a list of chemicals and broad endpoints from which to choose. Students were guided as to how to choose a specific endpoint and quantify it such that the project was feasible within a semester. Prior to beginning the research, they learned how to handle, transfer and identify the developmental stages of C. elegans, as well as write a brief literature review of any previous research with C. elegans and their chosen chemical. They then submitted, and ultimately revised, a research proposal. At the conclusion of their project, the pairs of students presented their poster to the campus community at a poster session, to which faculty, staff and administration were invited. Many community participants chose to contribute to the evaluation of the posters when provided a grading rubric. The experience allowed students to participate in the scientific research methods, including a literature review, research proposal and data analysis. Engaging students in these types of experiences encourages them to pursue a graduate education in toxicology. Some of the students involved in this project indicated they were interested in pursuing an advanced degree in toxicology; all suggested that this project was a valuable exercise in learning how to "do science."

PS 1873 DEVELOPING UNDERGRADUATE ENVIRONMENTAL HEALTH AND TOXICOLOGY CURRICULA WITH RESEARCH EXPERIENCE OPPORTUNITIES.

B. W. Brooks. *Baylor University, Waco, TX.*

Though toxicology degree programs are uncommon at the undergraduate level, environmental health majors represent an area of critical health need in the United States. For example, the Centers for Disease Control and Prevention report over 90% of the environmental public health workforce has no formal education in environmental health or public health, approximately 50% of the environmental public health workforce is eligible to retire within 5 years, and over 12,000 positions in environmental health remain unfilled. Presently, only 32 undergraduate environmental health majors are accredited by the National Environmental Health Science and Protection Accreditation Council (EHAC). Thus, the BS in environmental health curriculum at Baylor University was designed to meet such accreditation requirements, includes a number of courses in toxicology and risk assessment, and represents the second EHAC-accredited environmental health program in Texas, a state projected to double in population within the next 50 years. In addition to developing interdisciplinary curricula in toxicology and environmental health, undergraduates are often integrated in research teams and are required to participate in off-campus internships. Several innovative strategies implemented at the university level include First Year Seminars and Engaged Learning Groups for freshmen. These programs focus on specific topics and increase student retention in the sciences. As part of recent accreditation efforts for the Southern Association of Colleges and Schools, Baylor University developed a Quality Enhancement Plan (QEP) to expand research experience opportunities for undergraduates. This QEP developed the Undergraduate Research and Scholarly Achievement (URSA) Small Grants Program, an intramural funding mechanism. URSA funds are commonly used to offset costs associated with research supplies and travel to scholarly meetings, incentivizing incorporation of undergraduates in research groups by faculty mentors, who are recruited and retained with implicit expectations of undergraduate mentoring.

PS 1874 INTRODUCTION OF UNDERGRADUATE STUDENTS TO TOXICOLOGY-RELATED ISSUES THROUGH JOURNALING.

S. M. Ford. *Toxicology Program, St. Johns University, Queens, NY.*

Undergraduate students who study toxicology have a great interest in learning about the scope of the discipline and its relevance to their future. Toxicological problems and events are well-represented in the news; however, the shift of young adults away from traditional news souces over the past few decades is well-documented. According to D. Mindich (2005), one way to increase young people's interaction with news media is to convey the expectation that they should be conversant in current events. Journaling, which involves writing and reflection on events and/or experiences, is a way to integrate student interest in toxicology with active learning of current events in toxicology. Active learning is a pedagogical technique which appends thinking and reflection to learning activities. As part of the requirements for the course "Current Issues in Toxicology" in the B.S. program at St. John's University, students keep a journal of toxicology articles appearing in the news. Guidance is given for selecting sources and format of citations. Rather than being assigned readings by the instructor, students survey various news sources on their own and select three items per week. For each article, they write both a summary and an analysis to develop writing and critical thinking skills. At various times during the semester students choose articles from their journals to discuss in class. The journal represents 15% of the final grade and evaluation is based on presentation, suitability of articles, and quality of summary and analyses. Additionally, students are expected to use their journals as a source of examples for essay exams. Benefits of the journals include self-directed exploration of news sources, promotion of student knowledge of toxicology in the news, personal reflection on toxicological issues, enhanced writing skills through weekly practice, and development of critical thinking through analysis and discussion.

PS 1875 TOXLEARN: AN NLM-SOT EDUCATIONAL COLLABORATION.

P. Wexler[1], M. Kamrin[2], B. Eidemiller[3] and S. Ray[4]. *[1]Toxicology and Environmental Health Information Program, National Library of Medicine, Bethesda, MD, [2]Center for Integrative Toxicology, Michigan State University, East Lansing, MI, [3]Society of Toxicology, Reston, VA and [4]Pharmaceutical Sciences, AMS College of Pharmacology & Health Sciences, Long Island University, Brooklyn, NY.*

ToxLearn is a multi-module online Flash-based presenting the fundamentals of toxicology. It is a joint project of the Society of Toxicology (SOT) and the National Library of Medicine's (NLM) Toxicology and Environmental Health Information Program, with software development and production support from the Patient Education Institute. ToxLearn can be used as an undergraduate teaching tool to supplement course work, to give NLM users better insight into the scientific content of its databases and, more generally, to educate the public. It is a revision of NLM's ToxTutor, which will be phased out when all modules are complete. ToxTutor's 3 longer modules will be replaced by 8 or more shorter modules to retain its full scope while updating and enlarging with new developments in toxicology. It offers more graphics, interactivity, audio, a printable PDF version and a process for issuing certificates of completion. ToxLearn incorporates the International Union of Pure and Applied Chemistry's Glossary of Terms in Toxicology and makes frequent use of quizzes. A Steering Team of toxicologists and representatives from SOT and NLM are responsible for creating the content. Module I (Introduction to Toxicology and Dose Response) was extensively beta tested and became publicly available in January 2010, and has been very well received by users since then. Additional modules are in development.

PS 1876 SO YOU WANT TO START A TOXICOLOGY GRADUATE PROGRAM?

A. de Peyster. *Graduate School of Public Health, San Diego State University, San Diego, CA.*

Lessons learned about creating a toxicology graduate program are offered for those considering the challenge. Discussion addresses objectives, approaches, key findings, and other implications Important keys to success in our case were people with great interest and determination, appropriate expectations, and flexibility to address changing student demographics and need for education and skills training in specific areas. Because of some circumstances unique to the author's institution, discussion focuses on master's level programs, but basic principles can also be applied to doctoral studies. Benefits and challenges of affiliation with a school of public health are also explained along with tips for success applicable to other academic settings. Perceived need had already been identified before the author was recruited to develop a full curriculum. Realities were a different matter, however, and determined how the curriculum was oriented initially and evolved later. A critical mass of human resources was also identified during the initial years. Participation by other toxicologists in the region greatly enhances a program. As lab equipment was collected and campus lab spaces were being renovated, including a small vivarium, early students expected to gain proficiency in basic toxicology lab skills could be accommodated in other locations. Students always benefit from exposure to research in other campus or industry laboratories with different state-of-the art equipment. Core classes, lab and thesis requirements may be standard, but every student's needs

and career aspirations are unique. Appropriate flexibility within university regulations is encouraged. Conference participation is also strongly encouraged, as is publishing masters thesis data, although this is not required. Sufficient intramural and extramural funding are obviously always important for maintaining lab-intensive research training programs; however, our approach is founded in the belief that dedicated people with ingenuity can often make up for a lot of unpredictability and shortfalls in financial support.

PS 1877 TRISK: A EUROPEAN ADVANCED RISK ASSESSORS TRAINING PROGRAMME FOR TOXICOLOGY EXPERTS.

A. Moretto[1, 2], C. L. Galli[1], H. Hakansson[3], S. Price[4], B. J. Blaauboer[5], R. Kahl[6] and M. Gerevini[7]. *[1]University of Milano, Milano, Italy, [2]ICPS, Ospedale Sacco, Milano, Italy, [3]Karolinska Institute, Stockholm, Sweden, [4]University of Surrey, Guildford, United Kingdom, [5]Universiteit Utrecht, Utrecht, Netherlands, [6]Universitatsklinikum Dusseldorf, Dusseldorf, Germany and [7]Tecnoalimenti, Milano, Italy.*

A survey conducted in EU indicated that there is a need for trained risk assessors familiar with the current legislative framework in Europe. In this context, the EU-funded project TRISK has as its general objective the development of a training programme in risk assessment based on harmonised European criteria. These should be easily adoptable by institutions across Europe, and should focus on risk assessment methodology and procedure. TRISK fills an important need in the training of toxicologists for risk assessment by establishing a clear definition of training criteria and a recognition mechanism to qualify risk assessors. In addition, this project is aimed to fill the lack of training schemes and provide opportunities for practical, on the job training on chemical risk assessment for humans to young scientists or graduates in toxicology, as well as trained and expert toxicologists attracted by the opportunity to serve as member of the various scientific committees that deal with risk assessment in regulatory, industry and governmental bodies. Twenty-five participants have been enrolled from 22 European countries and the thought part of the course will be concluded in early 2011. This consists of 8 one-week modular courses addressing the main issues in chemical risk assessment. During 2011 the on-the-job training will take place and the final exam will be at the end of 2011. It is hoped that TRISK will contribute directly to the training of risk assessors across Europe in order to satisfy the constant needs for trained scientists to serve in the Commission Scientific Committees and ensure the sustainability of the EU risk assessment advice structure. It will also indirectly meet the needs of industry and the private sector who also require trained risk assessors.
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PS 1878 REGISTRATION OF SPECIALISTS IN TOXICOLOGY AND RISK ASSESSMENT IN AUSTRALASIA.

P. Di Marco[1] and B. Priestly[2]. *[1]Environment, Golder Associates, West Perth, WA, Australia and [2]Australian Centre for Human Health Risk Assessment, Monash University, Clayton, VIC, Australia.*

The Australasian College of Toxicology & Risk Assessment (ACTRA) was established in 2006 to provide for the professional development and continuing education of toxicologists and professional scientists engaged in health risk assessment (HRA). One of the ACTRA programs has been to establish a Register, so that the credentials and expertise of those practising in the fields of toxicology and HRA can be recognised. For toxicologists and health risk assessors to achieve and maintain the highest standards in their professional practice, they must be well trained, experienced and productive. It is therefore appropriate to recognise the achievement of such high standards with some form of accreditation or certification program. Such recognition implies professional competence and may assist aspiring and dynamic toxicologists to pursue a more clearly defined career path. Indeed, some of our toxicologists have already sought such recognition through one of the accreditation programs developed overseas (e.g. DABT, FATS). The basis for the ACTRA Register and the Code of Professional Conduct was established under an ACTRA By-Law, enacted in October 2008. The model for establishing eligibility for entry onto the Register is similar to that established by the British Toxicology Society and Academy of Toxicological Sciences and it accords with recommendations from IUTOX for establishing accreditation programs. Applicants must be vetted by a Registration Tribunal whose expertise and reputation in toxicology and risk assessment is of the highest order. A number of eminent toxicologists who have achieved such recognition in the international arena have agreed to serve on the Registration Tribunal. Once approved, registrant details will be publicised on the ACTRA website. Registration will be reviewed on a five year cycle subject to registrants meeting continuing education objectives in toxicology and RA, such as attendance at relevant scientific and educational activities, and participating in RA projects and activities and regulatory activities.

PS 1879 RESULTS OF THE 2010 SOCIETY OF TOXICOLOGY GLOBAL JOB OUTLOOK SURVEY.

J. Kerzee[1], M. Genter[2], J. A. Popp[3], R. D. Storer[4] and V. Vasiliou[5]. *[1]Health and Life Sciences Global Business, Battelle Memorial Institute, Columbus, OH, [2]Department of Environmental Health and Safety, University of Cincinnati, Cincinnati, OH, [3]Stratoxon, Lancaster, PA, [4]Laboratory Sciences and Investigative Toxicology, Merck & Co., Inc., West Point, PA and [5]Department of Pharmaceutical Sciences, University of Colorado, Aurora, CO.*

The current global economic crisis has created uncertainty in the job market, specifically in regards to the scientific sector. Preliminary data gathered from the US BioScience Sector-2010 Sub-sectional Report (Venn Research, 2010) indicate that toxicologist held approximately 117,000 jobs in 2010, an increase of 3.46 percent from 109,000 jobs held in 2008. Furthermore, according to the Bureau of Labor, the number of toxicologist jobs is projected to grow to over 153,000 by the end of 2018. In order to address questions around the market sector, global location, and scientific training required to obtain these projected jobs, the SOT Professional Needs Assessment Task Force (PNATF), in cooperation with the Career Resource and Development Committee (CRAD) and the Data Task Force, created the SOT Job Market Subcommittee. The Subcommittee was charged with developing a survey for the SOT membership, specifically those involved in the hiring toxicologists to 1) identify areas of growth and contraction in the global marketplace within the next 3–5 years; 2) identify critical training needs for practicing and future toxicology jobs in a global market; and 3) identify the current, and future, tools employers use to identify toxicologist with the necessary specialized training tools in the global marketplace. The data acquired from this survey will help toxicologists throughout their careers assess job opportunities, training needs, and world wide job opportunities. The results of this survey presented herein will complement the 2009 Professional Needs Assessment Survey and provide guidance to CRAD so that they may develop future educational programs and professional development opportunities for toxicologists.

PS 1880 PLACING TOXICOLOGICAL SCIENCE IN THE CONTEXT OF HISTORY, SOCIETY, AND CULTURE.

S. Gilbert[1] and P. Wexler[2]. *[1]INND, Seattle, WA and [2]National Library of Medicine, Bethesda, MD.*

Civilization has been shaped by the discovery and use of toxicological information. Take for example Socrates, who died in 399 BCE from eating a cookie made from hemlock. Or the many assassins who used arsenic to dispatch political rivals or annoying spouses, which finally stopped when Englishman James Marsh (1794-1846) developed a test for arsenic in the blood. The foundations of toxicology were created by a wide range of fascinating people who continue to influence us today. To explore the richness of toxicological history and provide a door for students to enter the world of toxicology, we have created the Toxicology History Room (THR). The purpose of the THR is to educate and inspire the viewer to explore the many ways that toxicology contributes to a sustainable and prosperous society. The THR consists of an expanding series of posters created by toxicologists with an interest in history and education. These posters strive to put toxicology in the context of history, society, and culture, and they also allow readers to not only explore toxicology but to examine relationships with fields such as chemistry, physiology, pharmacology, history, and sociology. The THR is a traveling exhibit that was most recently displayed at the IUTOX meetings in Barcelona, Spain and at the North American Congress of Clinical Toxicology meeting in Denver, Colorado. Most of the posters, with permission of the authors, are accessible online at www.toxhistoryroom.org as a project of Toxipedia/INND. The THR includes a look at individuals such as Mithradates the Great, Alexander the Great, and Ferreira da Silva, and examines events such as Love Canal, Bhopal, and the history of opium and of different toxicology societies. We invite you to explore and contribute to the THR or even display parts of the THR at your event.

PS 1881 ZEBRAFISH BEHAVIOR IN TESTS OF SOCIAL PREFERENCE AND SOCIAL NOVELTY FOLLOWING ACUTE PARACETAMOL, 5-HT_{1A} OR CB_1 AGONIST EXPOSURES.

P. A. Barba-Escobedo[1, 2] and G. G. Gould[2, 1]. *[1]Arts & Sciences, Texas A&M San Antonio, San Antonio, TX and [2]Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Psychiatric disorders such as autism may be linked with drug over-exposures in genetically or physiologically sensitive individuals during brain development. For example, juveniles who are administered substantial quantities of acetaminophen (paracetamol) and in combination with certain low-functioning enzymes to break

down the doses may produce more toxic metabolites. This can cause neuron damage and therefore lead to behavioral changes. The zebrafish (*Danio rerio*) can be used to test toxicological properties of compounds with more translational relevance than supplementary non-mammalian models given that it is a vertebrate. Because zebrafish mature rapidly, they are also beneficial for studying long-term effects of exposure to compounds in the juvenile stage. This pilot study was conducted to examine the effects of acetaminophen in comparison to the CB1 receptor agonist WIN 55,212 or 5-HT *1A* partial agonist buspirone on social behavior, also known as shoaling tendency, in zebrafish. Fish were exposed by bath to either 1 g/L acetaminophen, 1 mg/L buspirone or 0.1 mg/L WIN 55,212 that were dissolved in 200µl DMSO or home tank water (+/- DMSO) in a volume of 250 ml contained in a 600 ml beaker for 15 minutes. Fish were then placed in a social interaction and novelty test for 10 minutes each following 20 minutes of acclimation to test the effect of these drugs on shoaling tendency. We found that WIN 55,212, acetaminophen and buspirone all increased shoaling tendency, which is consistent with their targeting of neural circuits that might be involved in autism and low social interaction. Future studies will involve exposure of young-juvenile zebrafish to these same compounds and testing as adults.

PS 1882 TOXICITY EVALUATION OF INHALED LOXAPINE IN THE RAT.

B. E. Stewart[1], M. Garley[1] and A. Koester[2]. [1]*Alexza Pharmaceuticals, Inc., Mountain View, CA and* [2]*Battelle, Columbus, OH.*

The potential toxicity of inhaled loxapine was evaluated in male and female Sprague Dawley rats. Loxapine was delivered as repeated nose-only inhalation doses for 14 days followed by a 14-day, posttreatment recovery period (n=10/sex/group main study, n=5/sex/group recovery). Toxicokinetics was evaluated in additional animals (n=3/sex/timepoint). Mean daily dose levels were 1.7, 6.4 and 13 mg/kg for males and females combined (10 minute daily exposure). A separate placebo group of animals had the same dosing schedule. All animals survived until scheduled necropsy. Dose related CNS clinical signs were consistent with the extended pharmacology of loxapine, an antipsychotic with D_2 dopamine receptor antagonist activity. Treated animals in the mid and high dose groups showed significant gender-specific decreases in mean body weights and weight gain patterns compared to animals in the control group; weight decreases were considered secondary to lethargy and decreased activity. Histological changes related to inhalation exposure included squamous metaplasia of the larynx, which was considered a reversible, non specific effect due to particle impaction. Changes related to the extended pharmacology of loxapine at these doses included mammary hyperplasia in both sexes, and ovarian follicular cysts and mucification of vaginal epithelium in females. These histological reproductive tissue effects were anticipated based on the pharmacology of loxapine and the reported results of oral exposure studies in rats. Mammary hyperplasia in males and ovarian follicular cysts in females were no longer seen by the end of the 14-day posttreatment period, and the incidences of the remaining reproductive tissue changes were markedly reduced, indicating ongoing recovery by Day 29 of the study. Based on the persistence of the clinical signs until the morning following dosing, the slow recovery of body weight decreases and the partial recovery of the histological findings at the high dose, the no-observed-adverse-effect level (NOAEL) was considered to be 1.7 mg/kg/d, the low dose in the study.

PS 1883 TOXICITY EVALUATION OF INHALED LOXAPINE IN THE DOG.

M. K. Garley[1], B. E. Stewart[1] and R. Matheson[2]. [1]*Alexza Pharmaceuticals, Inc., Mountain View, CA and* [2]*Charles River Laboratories Preclinical Services Montreal, Senneville, QC, Canada.*

The potential toxicity of inhaled loxapine was evaluated in male and female beagle dogs. Loxapine was delivered as repeated oral inhalation doses for 28 days followed by a 14-day, posttreatment recovery period (n=3/sex/group main study; n=2/sex/group recovery phase for placebo control and high dose groups only). Toxicokinetics of loxapine and active metabolite 7-OH-loxapine was evaluated in all groups. Mean daily dose levels were 0.12, 0.95 and 1.8 mg/kg for males and females combined (11-18 minute exposure daily). All doses are expressed as loxapine free base and dose calculations assume a deposition fraction of 1. A separate group of animals received the placebo control with the same dosing schedule. All animals survived until scheduled necropsy. Treatment-related CNS clinical signs were observed in all loxapine treatment groups and included decreased activity, lying on side, weakness, tremors and/or uncoordination. Incidence and severity of these observations was dose-related and consistent with the pharmacology of loxapine, an antipsychotic with D_2 dopamine receptor antagonist activity. Incidence, severity and duration of clinical signs decreased with repeated exposure. There were no treatment-related changes in body weight. Slight decreases in food consumption noted throughout the treatment period were considered related to decreased activity following treatment and not considered of toxicological significance. There were no treatment-related changes in clinical pathology or immunoglobulins and no treatment-related ocular or cardiac changes. No treatment-related organ weight, gross or histological changes to the respiratory system or other organ systems were observed during the main study or recovery phase. There were no treatment-related changes during the recovery phase. The no-observed-adverse-effect level (NOAEL) was considered to be 1.8 mg/kg/d, the high dose in the study. This dose corresponds to Day 28 loxapine plasma AUC_{last} values of 467 and 577 ng•h/mL in males and females, respectively.

PS 1884 DOXORUBICIN-INDUCED PLATELET PROCOAGULANT ACTIVITIES: AN IMPORTANT CLUE FOR CHEMOTHERAPY-ASSOCIATED THROMBOSIS?

K. Kim, S. Kim, K. Lim, J. Noh, S. Kang, O. Bae and J. Chung. *College of Pharmacy, Seoul National University, Seoul, Republic of Korea.*

The prothrombotic risk of doxorubicin (DOX) has been frequently reported, yet the exact mechanism underlying it has not been fully elucidated. Here, we report that DOX can induce the procoagulant activity in platelets, an important contributor to the thrombosis. In freshly isolated human platelets, DOX treatment induced the procoagulant activity as determined by phosphatidylserine (PS) exposure and microparticle generation while platelet aggregation was not affected. Consistently with this, DOX-treated platelets and generated microparticles increased the generation of thrombin significantly in concentration- and time-dependent manners. DOX-mediated procoagulant activation was determined to be from intracellular calcium increase and ATP depletion which resulted in the activation of scramblase and the inhibition of flippase, enhancing PS externalization ultimately. DOX-induced reactive oxygen species (ROS) generation was determined to underlie the procoagulant activation of platelets. Along with the increased procoagulant activity, apoptotic events were also induced by DOX treatment, such as the dissipation of mitochondrial membrane potential($\Delta\Psi$), cytochrome c release, mitochondrial translocation of Bax and caspase-3 activation. A caspase inhibitor, QVD can attenuate DOX-induced procoagulant activity significantly, suggesting the involvement of apoptotic pathways in the DOX-mediated procoagulant activation of platelets. Rat in vivo thrombosis models confirmed the prothrombotic effects of DOX through procoagulant activity, where DOX administration resulted in increased venous thrombus formation along with increased PS exposure and $\Delta\Psi$ dissipation, reflecting that DOX-enhanced prcoagulant activity of platelets may lead to increased thrombus formation, indeed.

PS 1885 TOXICOLOGY ASSESSMENT OF SHETA2, A NOVEL CHEMOPREVENTIVE AGENT, IN RATS AFTER 28-DAY ORAL ADMINISTRATION.

K. K. Kabirov[1], I. M. Kapetanovic[2], A. Banerjee[1], A. D. Zakharov[1], D. M. Benbrook[3] and A. V. Lyubimov[1]. [1]*University of Illinois at Chicago, Chicago, IL,* [2]*National Cancer Institute, Bethesda, MD and* [3]*University of Oklahoma Health Science Center, Oklahoma City, OK.*

The toxicity of SHetA2 and the functional status of the CNS were studied in Crl:CD (SD) rats after 28 days of gavage administration (0, 100, 500 and 2000 mg/kg/day). No mortalities were seen. Decreased activity in one male in the 2000 mg/kg/day dose group was observed on days 21 and 24. Body weight gains which were significantly decreased on day 8 in the 2000 mg/kg/day dose group resulted in a decrease in total body weight gains (statistically significant in females only). Both males and females in the 2000 mg/kg/day dose groups had decreased food consumption on days 1–8 and on days 15–22 (males only). These effects were not seen at the end of the study. FOB measurements (in week 4) revealed no signs of neurotoxicity. There were no statistically significant differences in clinical pathology parameters between the groups administered SHetA2 and the control group. Relative prostate weights were significantly decreased in the 2000 mg/kg/day dose group. Relative adrenal gland weights were significantly increased in the 2000 mg/kg/day females. No pathology or histopathology lesions were seen in the control and 2000 mg/kg/day dose groups. Following gavage administration, peak plasma levels were seen at the 2 hr time-point in the 100 mg/kg/day dose group (52.9 ng/mL) and at the 6 hr time-point in the 500 and 2000 mg/kg/day dose groups (195.8 and 246.3 ng/mL, respectively). Following single IV administration at 5 mg/kg, the peak plasma SHetA2 level was seen at the 5 min time-point (1827.7 ng/mL). PK analysis showed extremely low (< 1%) systemic bioavailability of SHetA2 after oral administration for all doses tested. The half-life of SHetA2 was short due to high systemic clearance. The dose at the level of 2000 mg/kg/day was considered as the LOAEL. The NOAEL for daily oral administration of SHetA2 by gavage to Crl:CD (SD) rats was 500 mg/kg/day. The study was performed under Contract No. N01-CN-43306 from the NCI.

PS 1886 CALCIUM CHANNEL BLOCKERS DISRUPT LEARNING IN AN INCREMENTAL REPEATED ACQUISITION PROCEDURE.

J. Bailey[1], Y. Lin[2], W. Ravis[2] and C. Newland[1]. [1]*Psychology, Auburn University, Auburn, AL and* [2]*Pharmacal Sciences, Auburn University, Auburn, AL.*

Calcium regulation has long been implicated in learning processes and compounds that affect this regulation may have detrimental consequences to normal cognitive functioning. Most studies have used drugs that act on ligand-gated calcium channels to investigate this issue. Here we emphasize L-type calcium-channel blockers (CCBs), which act on voltage-gated channels and are used clinically for the treatment of cerebral ischemia and hypertension (among other uses). Nifedipine, verapamil, and nimodipine were administered to mice performing an incremental repeated acquisition (IRA) procedure. This procedure requires that a mouse acquire a different response chain (e.g., left-right-back-left nosepoke) every day. A control procedure required that they perform the same chain every day. Ketamine, which antagonizes ligand-gated NMDA receptors on Ca++ channels, was also used. A range of doses was injected ip to BALB/c mice (N=8). We hypothesized that the learning task would be more sensitive than the performance task and that effects would occur at doses lower than those that produce non-specific decreases in response rate. Response-rate decrements and learning and performance deficits were seen with nimodipine at 3 mg/kg. Verapamil did not produce any behavioral effects even at 10 mg/kg. Nifedipine reduced response rates at 3 mg/kg, but no changes in learning or performance. Ketamine selectively impaired learning at 3 mg/kg and higher doses. Lethality in 1/8 mice occurred at 20 mg/kg of nimodipine and verapamil. Plasma levels of nimodipine ranged from 91.1 ng/ml to 888.1 ng/ml after approximetly 15 minutes from acute administration of 10 mg/kg. Although the CCBs have similar mechanisms of action, they differ from each other in their behavioral effects and differ as a group from ketamine.

PS 1887 APOPTOTIC MECHANISMS OF ARSENIC TRIOXIDE IN HUMAN PROMYELOCYTIC LEUKEMIA (HL-60) CELLS.

C. G. Yedjou and P. B. Tchounwou. *Biology, Jackson State University, Jackson, MS.*

Acute promyelocytic leukemia (APL) is a blood cancer that affects people of all ages and strikes about 1,500 patients in the United States each year. The standard treatment of APL has been based on the combined administration of all-trans retinoic acid and chemotherapy including anthracyclins and cytarabine. However, 10-20% of patients relapse, with their disease becoming resistant to conventional treatment. Recently the Food and Drug Administration has approved the use of arsenic trioxide (ATO) or Trisenox for the treatment of APL, based on clinical studies showing a complete remission, especially in relapsed patients. In a recently published study we demonstrated that ATO pharmacology as an anti-cancer drug is associated with its cytotoxic and genotoxic effects in human leukemia cells. In the present study, we further investigated the apoptotic mechanisms of ATO toxicity using the HL-60 cell line as a test model. Apoptosis was measured by flow cytometry analysis of phosphatidylserine externalization (Annexin V assay) and caspase 3 activity, and by DNA laddering assay. Flow cytometry data showed a strong dose-response relationship between ATO exposure and Annexin-V positive HL-60 cells. Similarly, a statistically significant and dose-dependent increase ($p< 0.05$) was recorded with regard to caspase 3 activity in HL60 cells undergoing late apoptosis. These results were confirmed by data of DNA laddering assay showing a clear evidence of nucleosomal DNA fragmentation in ATO-treated cells. Taken together, our research demonstrated that ATO represents an apoptosis-inducing agent and its apoptotic mechanisms involve phosphatidylserine externalization, caspase 3 activation and nucleosomal DNA fragmentation.

PS 1888 MM-302, A HER2-TARGETED LIPOSOMAL DOXORUBICIN, LIMITS DOXORUBICIN ACCUMULATION IN CARDIOMYOCYTES, AND ENHANCES DOXORUBICIN UPTAKE INTO TUMOR CELLS *IN VITRO.*

J. Reynolds[1], B. Hendriks[1], P. Lucker[2], K. J. Olivier[1], P. Zandstra[2], C. Niyikiza[1], J. Park[3], C. Benz[4], D. Drummond[1], U. Nielsen[1] and T. J. Wickham[1]. [1]*Merrimack Pharmaceuticals, Cambridge, MA,* [2]*University of Toronto, Toronto, ON, Canada,* [3]*University of California, San Francisco, CA and* [4]*Buck Institute, Novato, CA.*

Doxorubicin, although discovered more than 40 years ago, remains a highly effective chemotherapy for the treatment of breast cancer. Despite the proven efficacy, the therapeutic potential and use of doxorubicin is limited by potential cardiotoxicity. To address the safety and efficacy limitations of currently available anthracyclines, we have designed a new pegylated liposomal doxorubicin (PLD) formulation, MM-302, that targets doxorubicin to HER2-overexpressing tumor cells while sparing cells with low HER2 expression. Antibody fragments that bind to HER2 are coupled to the outer surface of a PLD formulation. Here we show that MM-302 specifically binds and enters tumor cells overexpressing HER2 with minimal uptake into normal cells such as cardiomyocytes which express low levels of HER2. BT474-M3 breast cancer cells overexpressing HER2 and embryonic stem cell-derived human cardiomyocytes were incubated with free doxorubicin, PLD and MM-302 to characterize the binding, uptake and cytotoxicity of the drugs. Cardiomyocytes rapidly took up free doxorubicin which resulted in increased cellular stress and cytotoxicity. Importantly PLD and MM-302 did not appreciably accumulate in cardiomyocytes with little or no effect on cardiomyocyte viability. Furthermore, specific accumulation of MM-302 is observed in BT474-M3 cancer cells to a greater extent than free doxorubicin and PLD. These results demonstrate MM-302 protects cardiomyocytes from exposure to doxorubicin and the associated undesired off-target effects, while providing significant specific accumulation of doxorubicin in the targeted tumor cells. These characteristics result in a molecule with the potential for increased efficacy, limited off-target toxicities and a wider therapeutic range, which may benefit potential patients.

PS 1889 MECHANISTIC INSIGHT AND SENSITIVITY OF HIGH-CONTENT SCREENING (HCS) OVER SINGLE-PARAMETER CYTOTOXICITY ASSAYS.

P. Walker[2], J. Mein[1, 2], M. Jacewicz[1, 2], J. Gilbert[1, 2], R. Annand[1, 2], H. Gill[2] and K. Tsaioun[1, 2]. [1]*Apredica, Watertown, MA and* [2]*Cyprotex, Macclesfield, United Kingdom.*

Adverse drug reactions (ADRs) are a major cause of attrition in preclinical and clinical drug development and are the most frequent reason for the withdrawal of an approved drug from the market. Consequently, cost-effective identification of mechanisms underlying potential ADRs early in the drug discovery process have substantial potential for improving outcomes for both drug discovery and for patients. ADRs can result from a variety of mechanisms in major organ systems such as the liver, heart, and kidneys, with hepatoxicity being responsible for 50% of all ADR cases. The biochemical mechanisms of drug-induced liver injury (DILI) are complex. However, by using a combination of screening methods in one multi-parameter assay, sensitivity is improved and a mechanistic insight is provided. Conventionally cytotoxicity has been evaluated by a range of individual methods such as DNA synthesis, new protein synthesis, glutathione depletion, Caspase-3 activity and membrane integrity. Using High Content Screening (HCS) multiple observations can be analysed simultaneously; here we measure mitochondrial membrane potential, cytochrome C release, membrane permeability and cell loss, giving a greater understanding of the cells biological response. Data generated by HCS are compared against a commonly used cell viability assays such as MTT or Neutral Red. HepG2 cells (hepatocyte derived cell line) were plated and treated with test compound for 72h and measurements of cell health as described above were measured utilizing a HCS analysis instrument and compared to cytotoxicity determined via MTT. The panel of cell health markers proved more sensitive to known cytotoxic agents (20-1000 fold greater sensitivity) compared to the MTT assay, and no false positives where detected. Routine use of this assay will allow the identification of compounds with potential ADRs, giving insight into the mechanism of toxicity. This high throughput assay will help early determination of DILI in the drug discovery process.

PS 1890 BIPHASIC REGULATION OF INTRACELLULAR CALCIUM BY GEMFIBROZIL CONTRIBUTED TO THE INHIBITING L6 MYOBLAST DIFFERENTIATION.

R. Dai[1, 3], A. Liu[1], J. Yang[1] and F. J. Gonzales[2]. [1]*School of Biosciences and Bioengineering, South China University of Technology, Guangzhou, China,* [2]*Lab. of Metabolism, National Cancer Institute, Bethesda, MD and* [3]*Zhongshan Pharmass Corporation, Zhongshan City, Guangdong, China.*

Gemfibrozil causes higher risk of myotoxicity than other fibrate drugs, which are commonly used lipid-lowering agents for dislipidemia. However, such mechanism is poorly understood yet. In this study, gemfibrozil, within its clinical concentration range, was used to reveal the regulation of intracellular calcium ([Ca2+]i) in L6 myoblasts and further inhibit their differentiation. Ca2+ probe Fluo-4 AM detected by laser scan confocal microscopy and flow cytometry were used to detect the [Ca2+]i in L6 cells. Inhibition of differentiation upon L6 myoblasts as well as toxic effect caused by gemfibrozil was also quantitatively evaluated. Resultantly, gemfibrozil in 5-100 mg/l could biphasicly regulate [Ca2+]i in L6 cells, where sustained reduction was observed when the concentration was above 50 mg/l. The inhibition potential of gemfibrozil in L6 differentiation was concentration dependent with maximal effect under 50 mg/l, as monitored by creatine kinase and differentiation index, re-

spectively. Below 100 mg/l of gemfibrozil led to no significant apoptosis or direct cyotoxicity during the differentiation of L6 myoblasts. Furthermore, inhibited differentiation by 50 mg/l of gemfibrozil were only partially recoverable. The regulation of [Ca2+]i and inhibition of differentiation of L6 myoblasts are well correlate with the gemfibrozil concentrations employed, and these are within the clinical application range. It is noteworthy that clinical exposure concentrations associated with mobilization of [Ca2+]i may modify more biological responses besides inhibiting differentiation. Information revealed in this study indicated the relevant mechanism for gemfibrozil-induced myotoxicity.

PS 1891 BINDING OF THE THERAPEUTIC ANTIBODY CP-751871 TO IGF-1R INDUCES PARTIAL AGONIST SIGNALING IN HUMAN AND MONKEY IMMUNE CELLS.

D. U. Lee and B. Jessen. *Drug Safety, Pfizer R&D, La Jolla, CA.*

Insulin-like growth factor receptor type 1 (IGF-1R) signaling is thought to play a pivotal role in cancer growth, progression, and resistance to certain anti-cancer therapies. Strategies are being developed to block IGF-1R as an anticancer treatment. CP-751871 (known as figitumumab) is a humanized monoclonal antibody which binds to the IGF-1R and antagonizes ligand binding and receptor signaling. In preclinical studies, CP-751871 showed significant antitumor activity as a single agent and in combination with chemotherapeutics. Binding of IGF-1R by CP-751871 induced downregulation of the receptor, a mechanism that in part contributes to the antagonism of the antibody. In a monkey toxicological study, treatment with CP-751971 showed decreased cellularity of the thymus and bone marrow, which is consistent with the target biology of IGF-1R. However there were toxicities such as inflammation and cell infiltration of the heart, eye, and joints, which are not entirely consistent with the mechanism of action of the IGF-1R pathway. To investigate this discrepancy, we sought out to further determine the pharmacology of this antibody. CP-751871 alone induces tyrosine-phosphorylation of IGF-1R and serine-phosphorylation of AKT kinase in human and monkey T cells, indicating partial agonist activity. CP-751871 in combination treatment with the IGF-1 ligand is strongly antagonistic in cellular assays, indicating the main mechanism of action of the antibody is to block IGF-1R signaling in the presence of ligand. To determine if partial agonist signaling can lead to a cellular response, PBMCs from human and monkey were treated with the antibody and DNA replication based on BrdU incorporation was assayed. CP-751871 is able to induce some S-phase entry of these immune cells, but not to the extent of IGF-1 stimulation alone. Taken together, our data show that CP-751871 induces partial agonist signaling in immune cells through binding of the IGF-1 receptor in both monkey and human cells. These results could potentially explain the immune-mediated toxicities observed from the preclinical studies.

PS 1892 A 5-DAY ORAL REPEAT-DOSE TOXICITY STUDY IN SPRAGUE-DAWLEY RATS TO EVALUATE THE TOLERABILITY OF AN S1P AGONIST.

C. Hurst, T. Sullivan and J. Clarke. *Biogen Idec, Cambridge, MA.*

Sphingosine-1-phosphate (S1P) analogs are compounds that modulate immune responses in animals and in clinical studies. S1P binds to and activates five different S1P receptors (S1P1-5). These receptors are G protein-coupled receptors that associate with one or more G proteins. Activation and down regulation of S1P1 receptors on lymphocytes sequesters lymphocytes in lymph nodes and prevents their release into systemic circulation, while the activation of S1P3 receptors promotes some of the undesired adverse events associated with FTY720 (bradycardia and bronchoconstriction). This pilot toxicology study evaluated the tolerability of a S1P1 receptor small molecule candidate (BIO-XX) when administered to Sprague Dawley rats. Female Sprague Dawley rats received daily oral doses of 100, 300 and 700 mg/kg BIO-XX for 5 days. There were 3 early decedents (1 in the 300 mg/kg and 2 in the 700 mg/kg); 2 of the 3 were related to gavage error. The early death (found dead) of 1 female at 700 mg/kg on Day 3 was considered related to administration of test article. Clinical observations that were noted among animals being administered BIO-XX included piloerection, rough coat, porphyrin staining, soft feces, hunched posture, cold to touch, salivation during dosing, and scraping of the mouth on bedding post-dose. A statistically significant decrease in mean body weight gain (- 13%) was observed for the 300 mg/kg group compared to concurrent controls on Day 5. In the 700 mg/kg group, 1/5 females exhibited a 20% loss in body weight by Day 3. A statistically significant decrease (-17%) in mean body weight gain was observed for this dose group compared to control. Macroscopic findings observed during the gross necropsy included distended stomachs and bloated caecums for animals at all dose levels. In conclusion, administration of BIO-XX resulted in significant decreases in body weight gain at nominal doses ≥ 300 mg/kg. The changes in body weight associated with administration of nominal doses ≥ 300 mg/kg for BIO-XX was considered adverse.

PS 1893 THERAPEUTIC EFFICACY AND SAFETY EVALUATION OF UC-II ALONE OR IN COMBINATION WITH GLUCOSAMINE AND CHONDROITIN IN ARTHRITIC DOGS USING PIEZOELECTRIC SENSOR-BASED GROUND FORCE PLATE.

R. C. Gupta[1], T. D. Canerdy[1], J. Lindley[1], B. A. Carroll[1], M. Konemann[1], C. Hendricks[1], M. Bagchi[2], D. Bagchi[2] and J. T. Goad[1]. [1]*Toxicology, Murray State University, Hopkinsville, KY and* [2]*InterHealth Research Center, Benicia, CA.*

The investigation was undertaken to evaluate therapeutic efficacy of undenatured type-II collagen (UC-II) alone or in combination with glucosamine hydrochloride (GLU) and chondroitin sulfate (CHO), and also to determine their tolerability and safety in moderately osteoarthritic dogs. Client-owned dogs, in four groups (n=7-10), were treated daily for a period of 150 days with placebo (Group-I), 10 mg active UC-II (Group-II), 2000 mg GLU + 1600 mg CHO (Group-III), and UC-II + GLU + CHO (Group-IV). On a monthly basis, each dog was evaluated for overall pain, pain upon limb manipulation, and pain after physical exertion using different numeric scales. Pain level was also measured objectively using piezoelectric sensors-based Ground Force Plate (GFP) for peak vertical force and impulse area. In addition, dogs were examined every month for physical, hepatic (ALP, ALT, and bilirubin) and renal (BUN and creatinine) functions.Based on observations, significant ($P<0.05$) reduction in pain was noted in Group-II, III and IV dogs. But using GFP, significant increases ($P<0.05$) in peak vertical force (newtons/kg body wt) and impulse area (newtons-sec/kg body wt), indicative of decrease in arthritis associated pain, were observed only in Group-II dogs. None of the dogs in any group showed changes in physical, hepatic or renal functions. Based on GFP data, arthritic dogs treated daily with UC-II showed marked reduction in arthritic pain with maximum improvement on day 150. UC-II, GLU, and CHO operate through different mechanisms of actions. All supplements were well tolerated and found to be safe over a 5 month period.

PS 1894 AUTOMATIC SINGLE CELL CALCIUM TRANSIENT ANALYSIS FOR CARDIOTOXICITY SCREENING AND PHARMACOLOGICAL TESTING.

F. Cerignoli[1, 2], D. Charlot[3, 4], R. Ingermanson[2], K. Wei[1], A. Savtchenko[2], J. H. Price[2, 3], P. M. McDonough[2] and M. Mercola[1]. [1]*Muscle Development and Regeneration Program, Sanford-Burnham Medical Research Institute, La Jolla, CA,* [2]*Vala Sciences Inc., San Diego, CA,* [3]*Quantitative Microscopy Laboratory, Sanford-Burnham Medical Research Institute, La Jolla, CA and* [4]*Bioengineering, University of California, San Diego, CA.*

High Throughput (HT) screening for cardio-active drugs and cardiotoxicity using physiological relevant cells is a bottleneck of the modern drug discovery process. Current HT electro-physiological recordings are based on engineered tumor cell lines, underestimating the complexity of cardiomyocyte excitability and physiology. More physiological testing are reserved for late-stage development, contributing to late-stage drug failures resulting in estimated cost of over US$ 2.55 billion annually. To obtain high throughput physiological measurements, we have developed a Kinetic Image Cytometer (KIC) for dynamic imaging and automated cell-by-cell analysis of intracellular probes and report its use measuring Ca2+ intracellular dynamic. The instrument electrically stimulates and records intracellular Fluo-4 fluorescence from hundreds of cardiomyocytes per well in 96-well plates. The associated software segments the images and measures fluorescence dynamic on individual cells, determining Ca2+ kinetic parameters for nuclear and cytoplasmic compartments and performing statistical analyses on the entire cell population or gated subsets. Experiments performed on human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes using Na+ channel blockers (TTX and lidocaine), hERG blockers (sparfloxacin, cisapride and mosapride) and calcium channel blockers (nifedipine, diltiazem and ryanodine) validated discrimination of pharmacological effects through HT dynamic calcium measurement. Post-fixation also enables direct histological correlation of expression markers to individual cell kinetics. This is the first report of a platform for automated and detailed high throughput physiological analyses of cardiomyocytes for basic research, as well as drug screening and cardiotoxicity testing.

PS 1895 A STRATEGY COMBINING HIGH-CONTENT SCREENING AND ZEBRAFISH LARVAE TO PREDICT HUMAN DRUG-INDUCED HEPATOTOXICITY.

N. Mesens, M. Steemans, F. Van Goethem and J. Van Gompel. *Global Preclinical Development, Janssen Pharmaceutical Companies of Johnson&Johnson, Beerse, Belgium.*

Drug-induced liver injury (DILI) represents a major challenge for pharmaceutical preclinical development as it signifies a major reason for pre-and clinical compound attrition and market withdrawn (30 registered drugs over the last 10 years). High

content cytotoxicity screening (HCS) on human HepG2 has been proposed as a valuable early screening model (applicable during the discovery phase) for the prediction of DILI in man (O' Brien; 2004). Here we show the in house validation of a 6-endpoint HCS-approach based on 99 reference compounds (biased between hepatotoxic and non toxic compounds). We could demonstrate a sensitivity of 72% towards the prediction of strong hepatotoxic compounds and 53% when all hepatotoxicants were included. Specificity of the assay remained 100%, a crucial prerequisite for any early cytotoxicity screening assay. To further improve the prediction of DILI during the discovery phase, we propose a combined strategy including the HCS and the phenotypic zebrafish hepatotoxicity assay. The simplicity of the HCS allows higher throughput screening, while the complexity of the zebrafish enables the detection of more complex mechanisms of hepatotoxicity at a later stage of the discovery phase. Here we show our evaluation of zebrafish larvae as an additional model to predict human hepatotoxicity. Although some shortcomings of the assay were identified (subjective endpoint, uptake issues and screening of one target organ toxicity only) the obtained predictivity was promising. Significant higher sensitivity indices were achieved, indicating the whole liver system of the zebrafish is able to detect more hepatotoxicants compared to the HCS, due to its higher level of complexity. Another important aspect of improving the prediction is the characterization of the reference compounds in function of their induction mechanisms of toxicity. With a subset of 45 compounds thoroughly characterized, we do succeed in obtaining significantly higher sensitivity indices.

PS 1896 MULTIPLEX DETECTION OF OXIDATIVE PHOSPHORYLATION AS A MECHANISTIC INDICATOR FOR DRUG-INDUCED MITOCHONDRIAL TOXICITY.

W. Zheng[1], L. Goretzki[1], G. Korzus[1], J. Wang[1], J. Murray[2] and R. Capaldi[2].
[1]R&D, EMD Millipore, San Diego, CA and [2]R&D, MitoSciences, Eugene, OR.
Sponsor: R. Wiese.

Oxidative phosphorylation (OXPHOS) produces more than 95% of the conserved cellular energy in the form of ATP under normal conditions. This process involves 5 different protein complexes, OXPHOS Complex I-V. The overall process of oxidative phosphorylation is tightly controlled by transcriptional, post-translational and substrate feedback regulations. Due to the dual genetic origins of OXPHOS enzymes from both nuclear and mitochondrial DNA, the mitochondrial DNA and its OXPHOS protein products are specifically susceptible to toxicity induced by a variety of drugs including antiviral and antibiotic compounds. Not surprisingly, the ability to monitor the levels of the 5 OXPHOS complexes need to be a key part of drug development and drug toxicity studies. However suitable high throughput approaches do not exist. Here we describe a high throughput, multiplex approach to monitoring OXPHOS complex I, II, III, IV, V and NNT using the Luminex xMAP technology. To evaluate the utility of the assay in the early detection of drug induced mitochondrial toxicity, HepG2 cells were treated with chloramphenicol, an antibiotic compound, ddC, an antiviral compound and two anti-diabetes drugs, rosiglitazone and troglitazone, one in widespread use but with safety concerns for cardiotoxicity, the other pulled from the market because of serious hepatotoxicity. The OXPHOS multiplex panel was used to quantify the OXPHOS complexes in the treated cells in comparison to the mock treated controls. The results indicated that the OXPHOS panel can effectively detect the mitochondrial toxicity and provides mechanistic insights into the drug induced cellular and organ damages at the OXPHOS expression level. The data suggest that OXPHOS multiplex assay could be used as a novel safety screen tool to identify potential off-target effects for new antiviral, antibiotic and other drug molecule leads.

PS 1897 IMPORTANCE OF RAT AND HUMAN SCREENS FOR ARYL HYDROCARBON RECEPTOR (AHR) ACTIVATION/CYP1A INDUCTION.

H. J. Garside, S. Atwal, M. R. Brown, J. Bowes, M. Graham and J. Valentin.
Global Safety Assessment, AstraZeneca, Macclesfield, United Kingdom.

Persistent activation of the aryl hydrocarbon receptor (AhR) by xenobiotics can lead to a wide array of toxicological responses including tumour promotion and endocrine disruption in both pre-clinical animal models and man. However, as there can be species specific differences between compounds that activate rat and human AhR, assessment of compound action on the AhR from both species allows better prediction of toxicological risk throughout drug discovery and development. Activation of the AhR can be detected by quantifying levels of the endogenous downstream transcriptional targets, CYP1A1 and CYP1A2 in both rat and human immortalised cell lines using commercially available antibodies. In this system the standard AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) produced an EC50 value of 5.4 pM in the rodent H4-II-E-C3 cell line and 167 pM in the human HepG2 cell line. The rodent PXR agonist/CYP3A inducer pregnenolone-16 alpha-carbonitrile (PCN) was used as a negative control compound and, as expected, did not produce a signal above control levels in either cell line.
Results from internal screening programmes show that 34% of compounds induce CYP1A levels in the rat cell line, 10% in both the rat and human cell lines and 2% in the human cell line alone. Results indicate that the rat cell line is more sensitive to AhR activators than the human counterpart and that IC50 values should be used as a factor in the risk assessment of this liability. Taken together these data can be used to predict and explain the mechanism of effects on the liver, immune and endocrine systems in preclinical safety studies in early and late development. Importantly, the identification of human specific AhR activators, whose potential toxicological effects would be missed in preclinical animal work, can be detected at an early stage of the drug discovery process.

PS 1898 NOVEL *IN VITRO* APPROACH FOR ASSESSING CARDIAC CONTRACTILE LIABILITIES USING MICROPATTERNED MUSCULAR THIN FILMS.

A. Grosberg[1, 2], M. D. Brigham[1], J. A. Goss[1], P. W. Alford[1] and K. K. Parker[1, 2].
[1]Harvard School of Engineering and Applied Sciences, Boston, MA and [2]Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA. Sponsor: A. Bahinski.

Cardiotoxicity is one of the major forms of toxicity seen in drugs and accounts for a major portion of drug recalls and delays experienced in regulatory approvals. In addition, recent regulation by the FDA has made assessment of cardiovascular safety a critical focus during the development of new antidiabetic therapies for Type 2 diabetes mellitus. It is therefore necessary to develop novel and better human-predictive screening assay systems in order to reduce attrition and increase drug discovery productivity. We have been able to microengineer functional heart tissues by culturing cardiomyocytes on one surface of elastomeric polymer thin films (muscular thin film; MTF) micropatterned with extracellular matrix proteins to promote spatially ordered, two-dimensional myogenesis. In this device, the MTFs remain secured at their base to a glass surface. During the contraction cycle the films bend up from the glass coverslips, and the degree of bending is directly correlated to the contractility of the cardiomyocytes. These heart tissue constructs are electrically functional and actively contractile, generating stresses comparable to those produced by native cardiac muscle. Cardiac muscle engineered with neonatal rat ventricular myocytes and paced at 0.5 Hz generated stresses of 9.2 ± 3.5 kPa at peak systole, similar to measurements of the contractility of papillary muscle from adult rats. These MTFs are amenable to incorporation into microfluidic channels and multiwell plates, providing a platform that uses a significantly smaller amount of cells and ability to multiplex the assay. This technology provides higher throughput analysis of anisotropic cardiac tissue contractility, which can be used to measure the cardiac contractile liability of drugs, biologics and nanotherapeutics.

PS 1899 A NOVEL *IN VITRO* APPROACH TO IDENTIFY MECHANISMS OF HEPATOTOXICITY.

C. Hu, D. Pietrzak, K. French and K. Frazier. *Safety Assessment, GlaxoSmithKline, King of Prussia, PA.*

Hepatocellular toxicity is a serious issue in drug development. Defining a mechanism can be difficult because of the numerous mechanisms involved and their overlapping temporal nature. We developed a multiparameter approach in HepG2 cells using both cytometric and biochemical techniques and verified endpoint sensitivity using various toxicants with discrete and well-defined mechanisms. Nuclear density, cell viability, mitochondrial membrane potential (MMP) and oxidative stress (OS) were monitored using HO33342, TO-PRO-3, TMRM and c-H2DCFDA, respectively, and quantified using laser scanning cytometry. Cells were exposed to carbonyl cyanide 3-chlorophenyharazone (CCCP), a known mitochondrial uncoupler, and menadione (MD), an OS inducer, for 2h and 24h. CCCP markedly depolarized mitochondria and unexpectedly increased OS in a dose-dependent manner with no effect on cell viability after 2h exposure and depolarized mitochondria with no effect on OS and minor effect on viability after 24h. In MD treated cells, increased OS and cytotoxicity were observed at both time points. Intracellular ATP concentrations were measured via bioluminescence and reduced glutathione (GSH) content was measured via flow cytometry using monobromobimane (MBBr) and known GSH depleter N-ethylmaleimide. Intracellular calcium ion levels were measured via flow cytometry using Indo-1 AM and levels rapidly increased after the addition of the toxicant ionomycin. Lipid peroxidation was measured via thiobarbituric acid-reactive substance quantification, which was increased with the addition of the toxicant cumene hydroperoxide. This novel multiparameter approach successfully evaluated multiple key mechanisms of hepatotoxicity and can easily be adapted to assessment of drug candidates during development.

PS 1900 GENERATION OF PRECISION CUT LIVER SLICES FROM PINCH BIOPSY SECTIONS.

K. Kowalkowski, A. Lisowski, W. R. Buck, E. Blomme and Y. Yang. *Abbott Laboratories, Abbott Park, IL.*

Precision-cut tissue slices retain the multicellular, structural and functional features of tissues and offers a more relevant approach to interrogate toxicity compared to cell-based in vitro techniques. This study examined the feasibility of generating viable tissue slices from biopsy-derived liver. In separate experiments, liver sections were removed from dogs and monkeys. One set of sections was removed using a traditional 8-mm coring tool, and the other set was removed using a pinch biopsy forceps, to replicate in vivo collection. For the traditional cores, slicing proceeded using standard methods. To facilitate slicing, the much smaller biopsy sections were first embedded in 2% agar to create 8-mm diameter plugs. Slices were cultured for 48 hours with 100 μM Rotenone as positive control for liver toxicant, with samples taken at 24 hours as intermediate time point. Supernatants and tissue homogenates (n=3) were analyzed for liver enzymes (ALT, AST and LDH) as endpoints for toxic effects. Slices were processed for histological evaluation. All vehicle-treated slices showed similar viability and low release for all three liver enzymes. In rotenone-treated slices there were differences between species and time points. In dog liver, histological examination showed rotenone-induced time-dependent hepatocellular necrosis correlating with significant release of enzymes. The severity was comparable in the biopsy and whole slices. In the monkey, there were no significant increases of enzymes at the 24 hour time point, but after 48 hours significant levels of all three enzymes were released from the traditional slices. For the biopsy slices, the only significant change was in the LDH levels. These data demonstrate the feasibility of using biopsy sections as a source of liver slices, which would enable toxicological evaluation without sacrificing animals, and justify further exploration and refinement of this technique.

PS 1901 A HUMAN BREATHING LUNG-ON-A-CHIP FOR DRUG SCREENING AND NANOTOXICOLOGY APPLICATIONS.

D. Huh[1, 2], B. D. Matthews[2], A. Mammoto[2], M. Montoya[1], H. Hsin[2] and D. E. Ingber[1, 2, 3]. [1]*Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA,* [2]*Children's Hospital Boston and Harvard Medical School, Boston, MA and* [3]*School of Engineering and Applied Sciences, Harvard University, Cambridge, MA.* Sponsor: A. Bahinski.

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 man. Here we describe 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. This mechanically active biomimetic microsystem represents valuable new model systems for in vitro analysis of various physiological functions and disease processes, in addition to providing low-cost alternatives to animal and clinical studies for drug screening and toxicology applications.

PS 1902 *IN VITRO* PREDICTION OF INJECTION SITE REACTIONS USING L6 RAT SKELETAL MUSCLE CELLS.

J. A. Willy[1], N. Schulte[2], E. Kreklau[1], J. Walgren[1], A. Stauber[1], J. L. Stevens[1] and T. K. Baker[1]. [1]*Toxicology, Eli Lilly and Co., Indianapolis, IN and* [2]*Preformulation, Eli Lilly and Co., Indianapolis, IN.*

Injection site reactions (ISRs) are commonly encountered in the development of parenteral drugs. Severe ISRs can lead to preclinical and clinical dose limiting toxicities. We developed an in vitro ISR screen to rank compounds for irritation risk, as a surrogate for ISRs, during early preclinical development. Reference compounds that were either known to induce ISRs or were non-irritating in vivo were used to validate this method. L6 rat myoblasts were allowed to form a confluent monolayer in 96 well plates followed by compound treatment for one hour at eight concentrations. Change in membrane integrity was measured using calcein-AM. Cells treated with non-irritants or non-irritating concentrations of compounds take up calcein-AM and convert it to fluorescent calcein which is detected using laser scanning cytometry. When membrane damage occurs, the fluorescent signal decreases in a concentration-dependent manner and comparison of concentration response curves allows for ranking of compounds. Using this assay, reference compounds were ranked in the following order (from highest to lowest irritation potential): diethylstilbestrol > mitoxantrone > mitomycin > doxorubicin > dacarbazine > vinblastine > cis-platinum > cyclophosphamide > sumatriptan succinate > metoprolol ≥ atenolol ≥ vasopressin ≥ ranitidine HCl. This rank order correlates with in vivo ISR potential for these reference compounds and, thus, demonstrates the usefulness of this assay in identifying potential irritants. While compound mediated ISR is a critical screening output for this model, it also has utility in understanding the role of dosing formulations in ISRs and the impact of formulation additives on shifting the ISR potential.

PS 1903 HEPARG CELL MODEL IN HIGH-CONTENT SCREENING ASSAYS GIVES MECHANISTIC INSIGHT INTO METABOLISM-MEDIATED HEPATOTOXICITY.

J. Mein[1, 3], P. Walker[3], M. Jacewicz[1, 3], R. Annand[1, 3], D. Steen[2], C. Chesne[2], H. Gill[2] and K. Tsaioun[1, 3]. [1]*Apredica, Watertown, MA,* [2]*Biopredic, Rennes, France and* [3]*Cyprotex, Macclesfield, United Kingdom.*

Drug-induced liver injury (DILI) is the most frequent reason for the withdrawal of an approved drug from the market, and it also accounts for up to 50% of cases of acute liver failure. Assays detecting mechanisms of potential adverse drug reactions (ADRs) allow early de-risking of drug discovery programs. High Content Screening (HCS) allows multiple mechanistic observations to be made simultaneously in a single cell; here we measure mitochondrial membrane potential, cytochrome C release, membrane permeability and cell loss. This multi-parameter assay gives mechanistic insight into the toxic insult on a cell. By conducting this assay in two human hepatocyte-derived cell lines, a further insight into the mechanism of DILI is provided, by identifying if the parent compound or reactive metabolites of the parent compound are potential hepatotoxicants. HepG2 cells are used as a surrogate model for liver toxicity (parent compound), whereas HepaRG cells are used to determine whether metabolites may be responsible for cell toxicity. Differentiated HepaRG is a metabolically competent cell line that displays many hepatocyte-like functions, including human CYP expression. HepaRG cells are preferred to primary human hepatocytes, which are hampered by the irregular availability, donor variability and scarcity of donors. Both HepG2 and HepaRG cells were treated with test compounds and indicators of cell health were measured with HCS instrument. Diclofenac, cyclophosphamide, and acetaminophen were chosen as compounds known to form reactive metabolites. HepaRG cells are found to be more sensitive at detecting the toxicity to the compounds with reported metabolism-based mechanisms of toxicities. This assay will allow the identification of compounds with potential ADRs and DILI, and identify compounds with metabolism-mediated toxicity risk

PS 1904 A NOVEL RESAZURIN/RESORUFIN CELLULAR ASSAY TO DETECT AND CHARACTERIZE REACTIVE ACYL GLUCURONIDES.

M. McMillian, J. Sasaki, M. Singer, F. Xu and H. Lim. *Johnson & Johnson, Raritan, NJ.*

Glucuronidation generally inactivates metabolites, making them more soluble and easy to eliminate. However, acyl glucuronides (AG) of some drugs are highly reactive, and may contribute to idiosyncratic reactions; NSAIDs provide many examples. Increased reduction of the redox dye resazurin to the more fluorescent resorufin was found to reflect reactive AG formation from many added parent compounds in hepatocyte and HepG2 cultures. The rank order of responses of compounds tested in this cellular assay at equimolar concentrations was somewhat different than previously reported following AG migration on glucuronidated compounds in KRH buffer. Responses ranged from very robust, such as diflunisal, flufenamic acid, and ethacrynic acid, to moderately robust, such as diclofenac, mycophenolic acid and flurbiprofen, to moderate, such as zomepirac and tolmetin, to mild or inactive, such as furosemide, fenofibrate, and valproic acid. Quantification of diflunisal and zomepirac AGs showed that more zomepirac AG was formed than

diflunisal AG (both less than 1% of parent), and the weaker response to zomepirac than to diflunisal thus could not be explained by different AG levels. Most of the very robust compounds were found to inhibit resorufin clearance (many due to acute cytotoxicity), and some of the moderate compounds were found to increase resorufin clearance relative to that in untreated wells. Borneol and β-glucuronidase increased basal resorufin formation, but had little effect on most compound AG-induced resorufin fluorescence. Together with established methods, this novel assay should allow further characterization of properties of reactive AGs in cells, and allow screening and avoidance of compounds with idiosyncratic effects.

PS 1905 HEPATOTOXIC DRUGS THAT DISTURB MITOCHONDRIAL FUNCTIONS ARE DETECTED IN THE SPERMATOZOA MOTILITY ASSAY.

I. Edebert[1], L. Löfstedt[1], I. Rafter[1], I. Cotgreave[1], P. Rigler[2], A. Serov[2] and R. Rigler[2]. [1]*Safety Assessment, Molecular Toxiclogy, AstraZeneca R&D Södertälje, Södertälje, Sweden and* [2]*Biophos SA, PSE-A EPFL, CH-1015, Lausanne, Switzerland.* Sponsor: P. Moldeus.

Mitochondrial toxicity has been implicated as a cause of drug induced toxicity in man. Hence, there is a need for a rapid and specific in vitro assay for the screening of drug candidate compounds in the early drug discovery process to sort out compounds that disturb mitochondrial function. The traditional methods to study mitochondrial functions are time consuming and based on freshly isolated mitochondria which require sacrifice of animal life.
In the present study, the motility of boar spermatozoa was used as an indicator of mitochondrial function. The movement of the spermatozoa tail is ATP dependent and the ATP is mainly produced by the mitochondria located at the base of the tail. Biophos SA has developed an instrument that combines an inverted microscope with a camera and a software algorithm, which calculates the speed and motility of the spermatozoa. Fresh boar sperm was obtained from a breeding facility on a daily delivery basis. The spermatozoa were incubated in 96-well plates for 30 minutes together with mitochondrial toxins or pharmaceutical drugs, before different motility parameters were monitored in the Biophos instrument.
The results show that the motility of the spermatozoa is reduced by low concentrations of the mitochondrial toxins rotenone, antimycin A, KCN and oligomycin. Also, the median IC50 value in a group of for pharmaceutical drugs that are known to affect mitochondrial functions was significantly lower than the median IC50s for drugs with other toxicity mechanisms and for non-hepatotoxic dugs. In conclusion, the Biophos spermatozoa motility assay may become a rapid and sensitive assay for detection of chemical compounds that affect mitochondrial functions, without the need to sacrifice laboratory animals or the use of expensive cell culture facilities.

PS 1906 IDENTIFYING KEY KINASES ASSOCIATED WITH BONE MARROW TOXICITY.

L. Marroquin, W. Hu and B. Jessen. *Pfizer, San Diego, CA.*

Bone marrow toxicity is one of the most common dose limiting adverse effects in drug development, especially for Oncology programs. For instance, it can lead to immunosuprression and often requires expensive growth factor treatment. It also may require dosing holidays and may limit treatment combinations. Many kinase inhibitors drugs have caused bone marrow toxicity. Kinase inhibitors may cause bone marrow toxicity through different mechanisms than traditional chemotherapy which could lead to synergistic effects. To identify the association between kinase inhibition and bone marrow toxicity, we performed siRNA mediated gene knock down experiments in human bone marrow mononuclear cells and CD34+ stem cells on several kinases that were implicated as key players in causing bone marrow toxicity. We first demonstrated this procedure on Jak2, which is a well characterized key kinase involved in hematopoietic signaling. It was previously shown that JAK2-deficiency is embryonically fatal in mice due to a lack of erythropoiesis. At 48 hour post electroporation, more than 70% knock down of Jak2 was confirmed at mRNA level using real-time RT-PCR in mononuclear cells and CD34+ stem cells. On day 6, this Jak2 specific siRNA resulted in more than 50% reduction in cell count in mononuclear cells as compared to scrambled control under exact electroporation conditions. In CD34 + stem cells that were induced to undergo differentiation to all lineages, there was a 30-40 % reduction in cell count, whereas CD34 + stem cells that were induced to undergo differentiation into a specific erythroid cell lineage led to a 50-60 % reduction in cell number. This model system can easily be used to identify key kinases associated with bone marrow toxicity. We currently looked at AXL, ALT, Jak3, AKT3; more kinases are under evaluation. This could be advantageous in the early stages of drug development where novel kinase targets can be assessed for potential bone marrow liability.

PS 1907 A RAT HEART SLICE MODEL TO EVALUATE THE IMPACT OF DRUG-INDUCED INJURY ON CARDIAC HEALTH.

J. Herrmann and A. Vickers. *Allergan, Inc., Irvine, CA.*

A novel model for studying drug induced cardiac injury employs precision-cut heart slices. This model facilitates the comparison of multiple compounds and increases flexibility of both drug concentration and time course, as compared to rat in vivo models. In addition, the slice model preserves the heart's multi-cellular complexity and aspects of three dimensional structure, offering advantages over in vitro co-culture models. Several drugs that impact cardiac health through a variety of mechanisms were tested in this model. These drugs included: adriamycin, an anticancer drug that can cause cardiotoxicity via free radical generation; isoproterenol, a beta adrenergic agonist commonly used as a model for cardiac ischemia; and etomoxir, an inhibitor of mitochondrial long-chain fatty acid oxidation that can cause cardiac hypertrophy. Heart slices (200 μm thick) from perfused hearts of adult male Sprague-Dawley rats were cultured for 24 hr with varying concentrations of each of the above drugs. Adenosine triphosphate (ATP) was used as a marker of cellular energy levels, and reduced glutathione (GSH) was used as a marker for oxidative stress. Preliminary results are as follows: treatment of heart slices with Adriamycin caused a concentration dependent (0-100 μM) decrease in both ATP and GSH levels; while Isoproterenol (0-50 μM) or Etomoxir (0-200 μM) each caused concentration dependent decreases in GSH levels. Heart slice gene expression profiles will be compared. From these findings, we conclude that the rat heart slice culture system is a successful model to examine the effects of cardiac injury caused by a variety of drug types. This model can be adapted for human heart to better predict drug effects in man.

PS 1908 DETECTION OF DRUG-INDUCED STRUCTURAL CARDIOTOXICITY IN THE RAT H9C2 CELL LINE: SUB-LETHAL ENDPOINTS OFFER GREATER SENSITIVITY THAN CYTOTOXICITY.

A. Pointon, S. Dawson, N. Gerges and J. E. Sidaway. *Global Safety Assessment, AstraZeneca, Macclesfield, United Kingdom.* Sponsor: D. Brown.

The aim of this work was to compare in vitro assays based on cytotoxicity and sub-lethal endpoints (fluorescent microscopy) to predict drug-induced structural cardiotoxicity during early safety screening. Nineteen drugs with clinical manifestation of structural cardiotoxicity and 16 non-cardiotoxic drugs were assessed. The rat H9c2 cardiac cell line and a non-cardiac cell line (HeLa cells) were incubated with 0.01-100μM of each drug. After 24h, ATP depletion was determined and IC50 values calculated from concentration-response curves (n =3). An IC50 of less than 100μM (mean 37 fold of clinical Cmax of each drug) was deemed to indicate human cardiotoxic potential and used to calculate sensitivity and specificity of each cell type to detect structural cardiotoxins. H9c2s were more sensitive to ATP depletion by cardiotoxins than HeLas (73.7% vs. 43.1%, both 100% specificity). 6h incubation with model cardiotoxins was found to cause minimal ATP depletion in H9c2s and the full drug panel was re-assessed at this time point for sub-lethal endpoints. Live cells were stained with fluorescent dyes for mitochondrial membrane potential (TMRE), mitochondrial health (mitotracker deep red), endoplasmic reticulum health (ER tracker), glutathione production (thio tracker), mitochondrial superoxide production (mitosox), calcium (fluo-4 AM) and nuclei count (hoechst 33342). Images were acquired with imageXpress (Molecular Devices) and processed using metaXpress; IC50 values were calculated (n=3). Within the sub-lethal parameters assessed, mitochondrial membrane potential and endoplasmic reticulum health were the most sensitive and, when used in combination detected structural cardiotoxins with 95% sensitivity and 100% specificity. These additional sub-lethal endpoints may provide a screening cascade to gain further mechanistic information. This approach may provide a screening strategy for in vitro detection of novel therapeutics that may cause structural cardiotoxicity.

PS 1909 *EX VIVO* CYTOCHROME P450 INDUCTION ANALYSIS AS A TOOL TO CLARIFY MECHANISM OF LIVER DYSFUNCTION IN RODENT TOXICITY STUDIES.

M. Graham[2], C. Garner[1], P. P. Lainee[1], H. Garside[1], A. Scally[2], H. Marshall[2] and J. Valentin[1]. [1]*Safety Assessment, AstraZeneca, Alderley Park, United Kingdom and* [2]*Safety Assessment, AstraZeneca, Charnwood, United Kingdom.*

Compound-dependent activation of intracellular receptors such as the aryl hydrocarbon receptor or members of the nuclear hormone receptor family are usually evidenced by toxicological responses including hepatocellular hypertrophy, decrease in plasma exposure after repeated administrations, changes in clinical pathology

biomarkers or observation of lesions at pathology in rodent studies. Cytochrome P450s (CYPs) are measured as a surrogate of receptor activation, facilitating improved interpretation of toxicity study findings. Therefore, liver samples were taken from preclinical rat dose range finding (DRF) and 28 day toxicity studies and analysed for levels of CYPs protein (CYP1A, CYP2B, CYP3A and CYP4A) by enzyme linked immunosorbant assay (ELISA) using commercially available antibodies. A review of 155 preclinical studies conducted between 2007 and 2009 and during wich CYPs were analysed showed that a compound dependent increase in one or more CYPs (> 3 fold above control) was observed in 26% of the studies. Among these CYPs positive studies, 67% showed increased liver weight (> 25% of control values), 32% showed increases in the liver function markers such as transaminases ALT or AST (> 30% of control values), 35% showed decreases in exposure (> 30% difference between last day and first day AUC) and 47% showed pathology findings in the liver. The relatively low incidence rate of CYP induction observed in the reviewed studies does not justify the inclusion of this endpoint as a routine analysis in toxicity studies. However, CYP induction analysis should be used when liver dysfunction is suspected from study findings as it can provide a valuable insign into the mechanisms of toxicity. This might facilitate the clarification of differences in species' sensitivity during preclinical development and the determination of relevance of these findings to man prior to clinical trials.

PS 1910 A FLOW CYTOMETRIC MULTIPLEX HIGH-THROUGHPUT APOPTOSIS ASSAY USING THE HTFC™ SYSTEM.

C. Black[1], B. Stout[1], K. Luu[1], T. Duensing[1], P. Rana[2] and Y. Will[2]. *[1]IntelliCyt Corporation, Albuquerque, NM and [2]Compound Safety Prediction, Pfizer Inc., Groton, CT.*

To determine the safety and efficacy of new drug targets, cell-based methodologies are routinely utilized to screen large compound libraries. However, traditional cytotoxicity assay methodologies often only provide single endpoints and single readouts. Here we describe a novel high-throughput flow cytometry (HTFC™) system that can rapidly provide biological data at single cell resolution in both 96- and 384-well plate formats. Using this system, we have validated a multiplex apoptosis screening assay that combines measurement of caspase 3/7 activation, cell viability, and cell counts from each well. The assay had both a robust assay window (>80 fold) and Z' values (> 0.7). Screening reproducibility was assessed using staurosporine and resulted in intra- and inter-assay variation of less than 23%. A total of 250 compounds were further investigated using the HTFC-based assay. These compounds were tested from 300 μM to 0.3 μM and incubated against Jurkat cells for 4 and 24 hours in a 384-well format. A 'necrotic response signature' was based on large changes in cell counts within the well, a significant drop in viability, and similarity to an apoptotic negative control such as Triton-X. We observed a variety of apoptotic and necrotic inducing behaviors, for example, simvastatin at 300 μM displayed a necrotic response signature similar to Triton-X based on a 10-fold drop in cell count and a 50% loss in viability. Other similar necrotic compounds at 300 μM against Jurkat cells at the 24 hour time point were: progesterone, atomoxetine, simvastatin, and tacrine. One clear example of an apoptotic compound through a caspase 3/7 mechanism was amsacrine which resulted in a dose response without an acute loss in cell count. The amsacrine EC50 values for caspase 3/7 activation were 12 μM at 4 hours and <0.3 μM at 24 hours. In summary, this method can rapidly identify apoptotic cells in a single read and simultaneously provide a 'necrotic response signature' for compounds indicating cell death through non-apoptotic mechanisms.

PS 1911 POLYSORBATE 80 14.7% IS A SUITABLE VEHICLE IN LONG-TERM RAT TOXICOLOGY STUDIES.

E. Lesage[1], M. Awori[1], F. Vlasseros[1], S. Y. Smith[1], T. Ahn[2] and K. Veverka[2]. *[1]Toxicology, Charles River Preclinical Services, Montreal, QC, Canada and [2]GTx Inc., Memphis, TN.* Sponsor: M. Vézina.

Polysorbate 80 (PS80) is a surfactant and emulsifier used in foods and in the manufacture of some medications for parenteral administration. PS80 is also a commonly used vehicle in preclinical toxicology studies. However, historical data on the use of PS80 in long-term rat studies is limited. The FDA Inactive Ingredients Database lists the maximum potency for oral suspensions of PS80 as 12.5%. PS80 is frequently used at concentrations up to 1.0% (w/w) in oral toxicology studies. The objective of this study was to evaluate the safety and tolerability of daily oral administration of 14.7% (v/v) PS80 for up to 26 weeks in Sprague-Dawley rats, a duration longer than has been previously documented in this species.
PS80 was diluted in deionized water to prepare a solution of 14.7% which was administered orally daily for 26 weeks to 40 male and 40 female Sprague-Dawley rats (Rattus norvegicus) aged between 12 to 13 weeks old and weighing between 301 to 464 g for males and between 231 to 284 g for females at the start of dosing. Animals were dosed at a dose volume of 5 mL/kg/day, 792 mg/kg/day, for up to 26 weeks. Twenty (20) rats/sex were dosed for 13 weeks, of which 15/sex were euthanized, and 20/sex were dosed for 26 weeks of which 15/sex were euthanized, with remaining animals retained untreated and euthanized after 4 weeks recovery.
The administration of 14.7% PS80 during a period of 13 or 26 weeks was not associated with any clinical signs or effects on food consumption, body weights, ophthalmology, clinical pathology and histopathology Based on these data, Polysorbate 80 was considered to be non-toxic at a concentration of 14.7% when administered daily by oral gavage to rats for up to 26 weeks and is considered a suitable vehicle for use in long-term rat toxicology studies.

PS 1912 FUNCTIONAL ASSESSMENTS IN REPEAT-DOSE TOXICITY STUDIES IN RODENTS AND NON-RODENTS: OVERCOMING PREJUDICES.

W. S. Redfern, L. Ewart, P. Lainée, S. Robinson and J. Valentin. *Safety Assessment UK, AstraZeneca R&D Alderley Park, Macclesfield, Cheshire, United Kingdom.*

In vivo safety pharmacology studies generally involve a single administration of a candidate drug, followed by functional measurements for up to 24 h. Whereas pharmacological responses tend to be fairly rapid in onset, and are therefore detectable after a single dose, some diminish on repeated dosing (tolerance), whereas others increase in magnitude (sensitization). Also, many toxic effects of drugs are either delayed in onset or require multiple exposures before they develop (e.g., neuropathy). Therefore, single-dose safety pharmacology studies may sometimes miss these late-onset effects, or underestimate the magnitude of an effect. Functional measurements can be incorporated into repeat-dose toxicity studies, either routinely (e.g., ECG) or on an ad hoc basis. Drivers for this are both scientific (based on the reasons outlined above), and regulatory, e.g., the safety evaluation of biotechnology products (ICH S6) and end-stage cancer therapies (ICH S9) does not necessarily require stand-alone safety pharmacology studies. There are inherent difficulties in achieving this: the availability of suitable technical and scientific expertise in the test facility; suboptimal laboratory conditions; use of simultaneous (as opposed to staggered) dosing; prioritisation of toxicokinetic sampling; unsuitability of certain techniques (e.g., use of anaesthesia; surgical implantation; food restriction); low portability of some equipment. Nonetheless, 'fit-for-purpose' data can still be acquired without requiring additional animals. Examples from our facility include assessment of behaviour, sensorimotor function, visual function, autonomic functions, ambulatory ECG, echocardiography, and respiratory function. This is entirely achievable if functional measurements are relatively unobtrusive, both with respect to the animals and the toxicology study itself. Careful pharmacological validation of any methods used, and establishing their detection sensitivity, is vital to ensure the credibility of generated data.

PS 1913 AN IMPROVED SYSTEM FOR SURGICALLY IMPLANTED IN-DWELLING CATHETERIZATION FOR LONG TERM INTRAVENOUS INFUSION IN CYNOMOLGUS MONKEYS, USING 2% TAUROLIDINE-CITRATE (TC) TO MAINTAIN CATHETER PATENCY.

R. Tavcar and K. Draper. *Lab. Research, Laval, QC, Canada.*

Extended intravenous (IV) infusion with surgically-implanted catheters is critical for non-clinical studies of many pharmaceuticals (e.g. anti-cancer agents or anti-infectives). A "catheter-in-catheter" (CIC) system provides many advantages over the traditional surgically implanted in-dwelling catheter. The CIC system requires only a single surgical procedure to insert the catheter into an appropriate vessel and employs a sub-dermal port system and fluid lock. For infusion/dosing, a second catheter is inserted through the port/initial catheter and can be connected to a swivel and jacket system. If desired, the second catheter and/or jacket can be removed between dosing events. The CIC system allows permanent external port access to a dosing vein and easy replacement of the removable infusion catheter while eliminating the need for repair surgeries. CIC can be used in conjunction with telemetry equipment or other physiological monitoring to capture pharmacodynamic effects that can be obscured by animal handling for manipulation of an in-dwelling catheter. To evaluate the feasibility of using 2% TC (a broad spectrum anti-microbial agent) in the fluid lock of the CIC system, one group of cynomolgus monkeys was infused continuously with sterile saline for 6 months, and a second group was infused for 24-hour cycles, every 2 weeks for 6 months. In the group that received bi-monthly infusions, TC from the catheter lock was flushed into the animals, and they were assessed for clinical pathology and/or toxicological effects at 24 hr post-flush following each cycle. There were no TC-related clinical signs or clinical chemistry changes in the animals. Thus, 2% TC appears to be a safe, feasible option for use in the fluid lock of the CIC system during long-term nonclinical studies in monkeys.

PS 1914 VALIDATION OF RESPIRATORY INDUCTANCE PLETHYSMOGRAPHY IN CONSCIOUS CYNOMOLGUS MONKEYS.

K. R. Kearney, M. R. Metea and P. R. Atterson. *WIL Research Laboratories, LLC, Ashland, OH.*

Non-invasive systems for acquisition of respiratory data are increasingly used for preclinical studies. Such systems allow for acquisition of respiratory data within standard toxicology study designs, to provide additional endpoints.
The aim of this study was to assess the feasibility of using respiratory inductance plethysmography (RIP) for continuous respiratory data collection from ambulatory animals, develop accurate calibration methodology and animal acclimation procedures. Methods: Three male and three female cynomolgus monkeys were acclimated to LOMIR infusion jackets and restraint chairs equipped with a helmet system including pneumotachograph (PNT) on 5 separate occasions. The RIP data were calibrated using known volumes derived from helmet and the PNT system. Following calibration of the RIP band system, animals were challenged with morphine and amphetamine at 5, 1 mg/kg, respectively, according to a crossover design. Respiratory data (frequency, tidal volume, and minute volume) were continuously collected for up to 24 hours. Respiratory parameters were analyzed with a repeated measure analysis of covariance to determine the sensitivity of the method in detecting respiratory changes. Multiple calibrations were performed using the PNT to develop accurate calibration procedures and determine drift of RIP signal over time. Results: Respiratory changes elicited with the reference compounds were detected with statistical analysis and followed the known time course of the drugs. RIP calibrations before and after the collection were comparable indicating that the drift was minimal over long term collections. Minimal acclimation of animals was determined to be 5 periods of restraint to the PNT collection system and 3 periods of jacket acclimation.

PS 1915 DRUG DISPOSITION AND CARDIAC TOXICITY: A LITERATURE-BASED REVIEW.

T. G. Hammond[1], J. Bowes[1], S. Boyer[3], M. P. Burnham[1], D. Cook[1], C. Gavaghan[3], M. Hance[4], M. M. Haywood[1], S. Matis[4], S. Roberts[1], B. Springthorpe[2] and J. Valentin[1]. [1]*Safety Assessment, AstraZeneca, Alderley Park, United Kingdom,* [2]*Safety Assessment, Astrazeneca, Charnwood, United Kingdom,* [3]*Safety Assessment, Astrazeneca, Molndal, Sweden and* [4]*Safety Assessment, Astrazeneca, Wilmington, DE.*

Cardiovascular safety liabilities remain a major cause of: drug attrition during preclinical and clinical development; adverse drug reactions; and withdrawal of medicines from the market. However, only a small proportion of these withdrawals are due to arrhythmias (~27% of 154 drugs). Compounds associated with cardiovascular toxicities show structural diversity that overlaps most of the "drug-like" space; therefore there is a need to look at potential mechanisms underpinning such toxicities. Bio-informatics and text-mining tools, enabling a thorough review of the literature, were used to assess the current situation. Cardiac accumulation of drugs, transporters and metabolising enzymes were found to have potential importance in cardiac drug toxicity. Many transporters (n=127) and P450s (n=14) are expressed, some selectively, in the heart; some of which are reported to transport (n=12) or metabolise (n=12) xenobiotics. Conversely, some drugs associated with cardiovascular toxicities are either transported (n=6) or metabolised (n=6) by these transporters / P450s, respectively. Most of these transporters and P450s have unknown functions in the heart; although genetic studies indicate that some have the potential to produce detrimental cardiovascular toxicities. Furthermore, the potential for drug-drug interactions exist but as yet these are unknown. Further research should be encouraged to understand the full impact of such observations.

PS 1916 POLYSORBATE 80 14.7% IS A SUITABLE VEHICLE IN LONG-TERM NON-HUMAN PRIMATE TOXICOLOGY STUDIES.

M. Awori[1], E. Lesage[1], F. Vlasseros[1], S. Y. Smith[1], T. Ahn[2] and K. Veverka[2]. [1]*Toxicology, Charles River Preclinical Services, Montreal, QC, Canada and* [2]*GTx, Inc., Memphis, TN.* Sponsor: M. Vézina.

Polysorbate 80 (PS80) is a surfactant and emulsifier used in foods and in the manufacture of some medications for parenteral administration. PS80 is also a commonly used vehicle in preclinical toxicology studies. However, historical data on the use of PS80 in long-term non human primate studies is limited. The FDA Inactive Ingredients Database lists the maximum potency for oral suspensions of PS80 as 12.5%. PS80 is frequently used at concentrations up to 1.0% (w/w) in oral toxicology studies. The objective of this study was to evaluate the safety and tolerability of daily oral administration of 14.7% (v/v) PS80 for up to 39 weeks in cynomolgus monkeys, a longer duration than has been previously documented in this species. PS80 was diluted in PRANG™ solution to prepare a concentration of 14.7% which was administered orally (cage side dosing) daily for 39 weeks to 10 male monkeys aged between 5 and 6 years old and weighing between 5.8 and 8.1 kg at the start of dosing. Animals were dosed at a dose volume of 4 mL/kg/day, 633 mg/kg/day, for the first 23 weeks and at 5 mL/kg/day, 792 mg/kg/day, for the remainder of the dosing period. Five monkeys were dosed for 13 weeks, of which 3 were euthanized, and 5 monkeys were dosed for 39 weeks of which 3 were euthanized, with the remaining animals retained untreated and euthanized after 4 weeks recovery. The administration of 14.7% PS80 during a period of 13 or 39 weeks was not associated with any clinical signs or effects on appetence, body weights, ophthalmology, electrocardiography, clinical pathology and histopathology. Based on these data, oral administration of Polysorbate 80 was considered to be non toxic at a concentration of 14.7% when administered daily to male monkeys for up to 39 weeks and is considered a suitable vehicle for use in long-term non human primate toxicology studies.

PS 1917 BACKGROUND DATA AND COMPARISON OF TWO LONG-TERM CONTINUOUS INTRAVENOUS INFUSION MODELS IN THE MOUSE USED FOR SAFETY ASSESSMENT STUDIES.

H. Van Wijk and A. Fraser. *Covance Laboratories Ltd., Harrogate, United Kingdom.* Sponsor: S. Kirk.

The need for improvements in the development of long term vascular infusion systems for rodents continues to increase. The mouse is proving a viable alternative to the rat but there is little published background data. This poster presents data on the comparison of two different techniques. Two continuous intravenous infusion techniques were conducted involving catheterisation of the vena cava via the femoral vein with different points of exteriorisation. The tail cuff technique, in which the catheter exteriorises via the tail stabilised by a tail cuff, was found to be appropriate for studies up to 28 days. However, for longer term studies a different continuous infusion model is needed and so the harness method was developed in which the catheter exteriorises via the dorsum, stabilised by a harness and skin button. Two 14 day and a 28 day tail cuff studies and a 91 day harness/skin button study (with a 7 day interim kill) were conducted. Data from these studies, including surgical success rates, reliability of the infusion method, bodyweights, clinical and behavioural observations, ophthalmoscopy, clinical pathology and histopathology were collected for comparison. For the tail cuff method, the reliability was 87.6% up to 14 days which dropped to 74% by 28 days due to pathology around the tail cuff. In terms of animal welfare, the tail cuff method allowed freedom of movement around the cage and normal sleeping patterns compared to the more restrictive harness/skin button method. The harness/skin button method was less reliable, mainly due to problems of securing the harness on the mouse. In addition, exophthalmos due to compression of the anterior tissues of the animals by the harness caused a keratitis in 75% of terminal kill animals. In conclusion, the tail cuff method is considered to be a reliable method for studies up to 28 days and acceptable with respect to animal welfare. After 28 days the harness/skin button method developed in this study is not yet considered to be a reliable alternative due to problems associated with the harness itself.

PS 1918 INTEGRATION OF PIG-A, MICRONUCLEUS, CHROMOSOME ABERRATION, AND COMET ASSAY ENDPOINTS IN A 28-DAY RODENT TOXICITY STUDY WITH 4-NITROQUINOLINE-1-OXIDE (4NQO).

M. McKeon, H. Chen, T. E. Lawlor, H. Murli, D. J. Roberts, A. Thakur, Y. Xu and L. F. Stankowski. *Covance Laboratories, Inc., Vienna, VA.*

As part of a multi-lab validation, we examined induction of Pig-a mutant red blood cells (RBCs) and reticulocytes (RETs) by flow cytometry during a 28-day subchronic toxicity study in male Sprague-Dawley rats using 4NQO (1.25, 2.50, 3.75, 5.00 and 7.50 mg/kg/day by oral gavage). Also evaluated were micronucleated RETs (mnRETs); blood, liver, and stomach DNA damage using the comet assay; and chromosome aberrations in peripheral blood lymphocytes. All genotoxicity endpoints were analyzed at two or more timepoints where possible. Mortality, body weight, food consumption, clinical pathology and organ weights also were analyzed, since this study was intended to evaluate integration of the genotoxicity endpoints into a standard 28-day toxicity study. Animals were sacrificed at Day 29, except those dosed at 7.50 mg/kg/day, which were sacrificed early (Day 15) due to extreme weight loss and morbidity/mortality. Dose-dependent body weight loss and/or decreased body weight gain reached significance ($p < 0.05$) at 5.00 and 7.50 mg/kg/day by Day 6 or 5, respectively. Observations at sacrifice included: de-

creased reticulocytes and lymphocytes, and increased neutrophils, at 7.50 mg/kg/day; increased reticulocytes, platelets, and neutrophils at 5.00 mg/kg/day; increased glucose levels at 3.75, 5.00 and 7.50 mg/kg/day; decreased total protein, globulin and albumin at 5.00 and/or 7.50 mg/kg/day; discolored glandular stomach and/or thickened nonglandular stomach at 5.00 and 7.50 mg/kg/day; increased stomach weight at 5.00 and 7.50 mg/kg/day; increased stomach:body weight ratios at 2.50 and 3.75 mg/kg/day; and increased brain:body weight ratios at >2.50 mg/kg/day. Significant, dose-dependent increases were observed for Pig-a mutant RBCs and RETs, and for mnRETs, but not for the other genotoxicity endpoints. These results demonstrate Pig-a mutation and other genotoxicity endpoints can be successfully integrated into subchronic toxicity studies, with potential reduction in animal usage.

PS 1919 COMPUTERIZED ARRHYTHMIA DETECTION IN LARGE TELEMETERED ANIMALS: A NOVEL STRATEGY TO ASSESS DRUG LIABILITIES.

R. Mikaelian[1], S. Authier[1, 3], F. Koeppel[2], F. Rossi[2], D. Labarre[2], S. Fournier[1] and E. Troncy[3]. [1]*Lab. Research Inc., Laval, QC, Canada,* [2]*Notocord, Paris, France and* [3]*Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, QC, Canada.* Sponsor: K. Draper.

A software application for arrhythmia detection was evaluated using control data from untreated telemetered Beagle dogs (with and without left ventricular pressure catheter) and cynomolgus monkeys. The technology was used to detect atrio-ventricular type II block, sinusal pause, atrial, junctional and ventricular arrhythmias. The computerized tool was used to evaluate at least 24-hour periods of continuous ECG data and all detections were subjected to semi-automated review (ECG trace evaluation from computer screen). All animals presented at least one incidence of premature atrial contractions, junctional complexes, premature ventricular contractions (PVCs) or atrio-ventricular (AV) blocks type II. Semi-automated review was used to confirm and classify the arrhythmias. In monkeys, the use of a DII configuration with a negative ECG lead inserted through the jugular vein and the positive lead sutured to the diaphragm close to the apex of the heart increased the signal to noise ratio and decreased artifacts. The incidence of ventricular arrhythmias was comparable in dogs with and without left ventricular pressure (LVP) catheters (0-5 PVCs per 24 hour period) in the limited population that was evaluated (n=5 with LVP and n=6 without LVP). As expected, the incidence of sinusal pause and AV blocks in dogs was higher than monkeys. The software application could be useful to increase efficacy of semi-automated ECG review obtained from telemetered animals and help quantify the incidence of arrhythmias.

PS 1920 HOW TO IMPROVE SENSITIVITY OF RESPIRATORY SAFETY PHARMACOLOGY STUDIES IN CYNOMOLGUS MONKEYS USING IMPEDANCE MONITORED BY IMPLANTABLE TELEMETRY: THE USE OF SUPER-INTERVALS.

F. Duguay[1], S. Authier[1, 2], S. Fournier[1] and B. Moon[3]. [1]*Lab. Research Inc., Laval, QC, Canada,* [2]*Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, QC, Canada and* [3]*Data Science International, St. Paul, MN.* Sponsor: K. Draper.

Continuous respiratory monitoring with implantable telemetry identifies circadian rhythm of respiratory parameters with lower variance during night time. This lower variance is expected to be associated with higher sensitivity to detect drug effects. The current study evaluated the effects of positive control drugs, medetomidine (0, 0.01, 0.02 and 0.04 mg/kg, IM) and buspirone (0, 0.03, 0.06, 0.12 mg/kg, IM) administered in a Latin square design at approximately 4:15PM on each treatment session to conscious cynomolgus monkeys (n=5). Analysis of variance (ANOVA) and a posteriori pair-wise comparisons using Dunnet's test was used with super-block analysis at 0-1 hour, 1-4 hours and 4-20 hours. The number and interval of post-dose timepoints were tailored to the pharmacokinetic profile of the drugs. Medetomidine resulted in a decrease in respiratory rate at all doses administered. Respiratory rate at peak effects was 37.6 breaths/minute (BPM) after saline treatment compared with 29.8, 29.0, 27.8 BPM at low, mid and high dose, respectively. At 15 min post-dose, minute volume was decreased by -9.5%, -17.0%, -21.8% at low, mid and high doses, respectively and the effects were present until 60 min post-dose. Buspirone induced an increase in respiratory parameters which were also dose-dependent. Dose administration prior to periods with lower intrinsic variability (late afternoon) may be a strategy to increase sensitivity. Tailored statistical analysis with selected timepoints focused on peak exposure presents another strategy to maximize sensitivity.

PS 1921 RESPIRATORY SAFETY PHARMACOLOGY MONITORING IN CONSCIOUS GÖTTINGEN MINIPIGS: QUALIFICATION OF A NEW MODEL.

C. Ponzi[1], J. Gervais[1, 2], E. Troncy[2] and S. Authier[1, 2]. [1]*Lab. Research Inc., Laval, QC, Canada and* [2]*Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, QC, Canada.* Sponsor: K. Draper.

Respiratory safety pharmacology is core component of non-clinical safety assessments as detailed in the ICH S7A guideline. The aimed of this study was to evaluate the feasibility of respiratory monitoring in conscious Gottingen minipig using pneumotachometer and data acquisition software for regulatory safety pharmacology. Six (6) Gottingen minipigs, three (3) males and three (3) females were used on this evaluation. Animals received a negative control drug (Saline 0.9%) and a positive control drug (Methacholine, 0.0034 mg/kg, 0.0135 mg/kg, 0.068 mg/kg) by intravenous injection using a Latin square design. Animals were acclimated to the respiratory monitoring procedure before the evaluation. Respiratory function was evaluated in conscious animals using a sealed mask and a pneumotachometer which was connected to the respiratory monitoring acquisition system. On each dosing occasion, respiratory function will be recorded continuously for a period of at least five (5) minutes before dosing and reported as baseline monitoring. After dosing, respiratory data was continuously recorded for at least 15 minutes. Parameters which were monitored included tidal volume, respiratory rate and minute volume. Baseline respiratory values in Göttingens minipigs were comparable to other laboratory animal species after allometric conversion with values of 26.78 ± 3.27 breaths/min, 203.37 ± 20.19 mL/breath and 4.66 ± 0.51 L/min. Dose-dependent respiratory changes were noted following methacholine administration in Göttingen minipigs with significant increases in respiratory rate, tidal volume and minute volume immediately after dosing. Based on our results, the Göttingen minipigs appears to be an adequate model for respiratory safety pharmacology when the use of this species is justified.

PS 1922 SUBCUTANEOUS LOCAL TOLERANCE FOR BIOLOGIC DRUG DEVELOPMENT: COMPARISON OF TWO APPROACHES.

S. Fleming-Weis[1], L. M. Diehl[2], D. Aleksandrowicz[1], J. Morrow[1], K. J. Zokowski[4], K. Ishida[1], L. E. Beebe[1], J. M. Pletcher[3], W. P. Davis[4] and J. B. Clarke[1]. [1]*Pharmacotoxicology, Biogen Idec, Cambridge, MA,* [2]*Toxicology, Charles River Laboratories, Spencerville, OH,* [3]*Pathology Associates, Charles River Laboratories, Frederick, MD and* [4]*Comparative Pathology, Biogen Idec, Cambridge, MA.*

Little regulatory guidance is available about subcutaneous route qualification for biologics drug development. Biologics are typically administered by the IV or subcutaneous routes and often, early nonclinical testing programs evaluate the feasibility of both routes. In some cases however formulation development cannot keep pace with nonclinical testing which must precede clinical development. Thus testing is either not conducted for technical reasons (insufficient concentration, stability, etc.) or may be repeated at a later time with a more mature formulation. Whether the objective of such testing should be largely to evaluate the effects at the local site of administration, or to provide longer-term bridging toxicology by an alternate route, is unclear from the available guidance. Unlike small molecules, for which administration by an alternate route (e.g. from oral to IV or SC) may significantly affect metabolic profile and thereby both pharmacological and toxicological profile, route changes for biologics primarily affect bioavailability and immunogenicity. Moreover, as immunogenicity in animals is considered of uncertain relevance to humans (see ICH S6) it is unclear whether observed changes in immunogenicity profile, would be interpretable from the perspective of assessing human risk. This poster reviews the available guidance on local tolerance testing, presents a summary of strategies taken by other companies using publicly available sources, and summarizes the results of two different local tolerance designs employed by Biogen Idec. Emphasis is placed on the overall objectives of local tolerance testing for the purposes of biologics drug development, and a study design is proposed that incorporates key areas of consideration for large molecules.

PS 1923 CYNOMOLGUS MONKEYS: FACTORS AND ISSUES FOR CONSIDERATION IN THE SELECTION OF SEXUALLY MATURE MALES AND FEMALES FOR PRECLINICAL SAFETY STUDIES.

D. Mitchell, H. Palmer, R. Davis and C. Chesher. *Safety Assessment, Huntingdon Life Sciences, Cambridgeshire, United Kingdom.*

Sexually mature cynomolgus monkeys are increasingly used for preclinical safety studies, particularly where medicines may affect reproductive organs. To ensure that the study objectives are met one must confirm completion of sexual maturation before dosing starts. It is generally accepted that using cynomolgus monkeys at least 4

years old should give a high probability of sexual maturity (1) but histopathology data at this laboratory has shown otherwise. This review of animal data from toxicology studies using sexually mature animals examines the parameters and factors involved in the determination of sexual maturation onset and completion. Discussions with suppliers, delivery, acclimatisation and pre-treatment investigations are included. The parameters reviewed include age, weight, physical characteristics of secondary development (e.g. descent and dimensions of testes, appearance of adult dentures), positive vaginal swabs and confirmed semen production and viability. Regular menstrual cycling, repeat viable semen sampling and assessment of hormone levels provide essential baseline data for intra-study/animal comparison, facilitating identification of toxicity and natural variability or progression of parameters. The results show that in isolation most of the parameters examined were open to interpretation as they were measured qualitatively. There was evidence that long-distance transport of the animals led to disruption of parameters measured at source. No individual parameter gave a clear determination of sexual maturity but repeated production of viable semen for males and a regular menstrual cycle for females provided the strongest evidence, accompanied by other parameters. Determination of sexual maturity in both sexes is a complex issue and many factors are involved. In conclusion, it is preferable to employ a weight of evidence approach using all available parameters to confirm sexual maturity in cynomolgus macaques.(1) Boyd Group Papers on the use of non-human primates in research and testing 2002 Paper 4.

PS 1924 SERIAL COLLECTION OF CEREBROSPINAL FLUID *VIA* A SURGICALLY OR PERCUTANEOUSLY IMPLANTED CATHETER IN CYNOMOLGUS MONKEYS.

J. K. Herman, R. Avery, M. Taschwer, P. Love, J. Fohey, M. Abdelhameed and W. Meier. *Nonclinical Safety Assessment, Covance Laboratories, Madison, WI.*

Serial Cerebral Spinal Fluid (CSF) collection in primates was evaluated for studies involving test materials that are active in the central nervous system (CNS). Although exposure can be confirmed with a single cisternal CSF collection, this single point evaluation does not allow assessment of time profiles of exposure and must be conducted in anesthetized animals. The purpose of this study was to evaluated two procedures for placing an indwelling catheter in the lumbar intrathecal space for collecting serial CSF samples from unanesthetized animals. The two models evaluated were 1) a surgically implanted catheter placed in the lumbar intrathecal space via hemilaminectomy technique and 2) a percutaneously implanted catheter placed in the lumbar intrathecal space via a "through the needle" catheterization technique. Animals with the hemilaminectomy were allowed at least 1 week post surgery for recovery while animals with the percutaneous catheter were allowed 24 to 48 hours for recovery. Animals were then given either a single dose of vehicle or a commercially available glycine transport inhibitor via oral gavage at a dose level of 2 mg/kg. Blood and CSF were collected predose and 1, 2, 4, 8 and 24 hours post dose. There were no clinical signs or changes in body weight or body temperature associated with test article administration or the surgically implantation of the catheters. The time to maximum levels (Tmax) for the test article in the CSF was similar to the plasma Tmax although exposure (AUC) was approximately 10 fold lower in the CSF than in the plasma. In general, exposure assessments between the two catheterization techniques were similar, although the percutaneous approach tended to yield slightly higher values. In conclusion, serial collection of CSF from conscious, restrained primates is a valid method for determining test article exposure in the CNS and allows for comparisons between plasma exposure, CNS exposure and potential clinical signs or pathology changes.

PS 1925 TEMPERATURE REGULATION AND THE ASSESSMENT OF TOXICITY.

K. L. Hastings[1] and M. D. Green[2]. [1]*Corporate Regulatory Affairs, sanofi-aventis, Bethesda, MD and* [2]*OVRR, DVRPA, U.S. FDA, Bethesda, MD.*

Temperature regulation is an important part of homeostatic regulation that influences many physiological and pharmacological systems. Factors involving body temperature involve both peripheral and centrally mediated physiological systems reflective of various classes of products making them potential responsive to a wide variety of drugs and biologicals. Changes in body temperature can be an important endpoint itself for example with certain classes of investigational products such as experimental vaccines. Additionally, body temperature may be a factor in the assessment of other endpoints of toxicological interest such as in vivo mutagenicity and exert on influence on the pharmacokinetics and pharmacodynamics of various drugs. Despite the potential value of measuring body temperature, this endpoint is infrequently determined in spite of the availability of sophisticated systems for its measurement. This presentation reviews the uses and tools presently available to examine body temperature for the assessment of toxicity.

PS 1926 APPLICATION OF THE DRIED BLOOD SPOT TECHNIQUE IN THE BIOANALYSIS OF SULPIRIDE.

J. Legrand, T. Ameller, R. Forster, R. Michaud and S. Laurent. *CIT, EVREUX Cedex, France.*

There has been much recent interest in the application of the Dried Blood Spot (DBS) technique in pharmacokinetics and toxicokinetics. In our facility, we have validated a bioanalytical method using the DBS approach for the determination of sulpiride in dog whole blood. Sulpiride is a second-generation antipsychotic molecule exhibiting bipolar functional groups. The DBS collection technique consisted of spotting 15.0µL droplets of whole blood samples containing sulpiride directly onto treated collection cards (GE-Whatman, DMPK cards type A®). After an appropriate drying time (at least 2 hours at room temperature), a small circular disc (3 mm wide) was punched out of the blood spot and immersed in methanol containing the Internal Standard (safinamide 0.20 µg/mL, 150 µL per sample). The samples were vortexed for 20 minutes at a moderate speed, the paper was removed from the solvent, and the extracts were evaporated (+35°C, 5 minutes) under a gentle stream of nitrogen. After reconstitution in organic solvent, the resulting mixture was transferred to LC-MS/MS for analysis (ESI positive mode). All the obtained results for sensitivity, linearity, reproducibility, selectivity and stability at room temperature fulfil the acceptance criteria described in current international guidelines. Specifically, no significant matrix effect or carry-over phenomenon was detected and precision and accuracy results were within ± 15%. Short-term stability was demonstrated after 69 hours of storage at room temperature and 114 hours at +4°C. A cross-validation between freshly prepared and overnight drying calibration curves and QC samples was also performed. Taking into account the practical advantages of the DBS technique (decreased number of required animals; lower collected blood volume; easier handling, shipping and storage), this method has been implemented for routine use in our facility for pre-clinical toxicocinetic/pharmacokinetic studies.

PS 1927 INTRAVITREOUS ADMINISTRATION IN THE RABBIT AND MINIPIG.

R. Forster, V. Haag, J. Legrand and B. Palate. *CIT, EVREUX Cedex, France.*

Intravitreous administration to the eye brings drugs into direct access with the retina, and there has been much recent interest in the clinical use of this route of administration in the therapy of macular degeneration and other diseases. For the preclinical development of these approaches, intravitreous administration to laboratory animals is required. Over recent years, we have performed several hundred intravitreous administrations to rabbits, minipigs and other species. The formulations to be administered must have appropriate characteristics, including attention to physico-chemical properties such as pH and tonicity. Critical points in the administration technique concern adequate preparation of the animals, the volume and rate of administration, needle gauge and sterile technique. Attention should be given to the frequency of the administration in order to avoid problems of tolerability. Finally, in a small proportion of administrations, adverse findings such as inflammation may result in clinical and histopathological findings. Electroretinography may be used to provide an indication that administration or reactions to administration do not have an adverse impact on functionality. In this poster we present the technique of administration that we have used in the rabbit and minipig, and data on background findings and their frequency. Overall, when appropriate precautions are taken, this route of administration can be used routinely for preclinical studies in the rabbit and the minipig.

PS 1928 ASSESSMENT OF COMMON PRECLINICAL VEHICLE FORMULATIONS USED IN DRUG DISCOVERY BY MAGNETIC RESONANCE SPECTROSCOPY (MRS).

R. Sriram and S. Liachenko. *Pfizer Inc., Groton, CT.* Sponsor: M. Paule.

To evaluate the effect of excipients in drug formulations MRS was utilized to monitor acute changes in creatine(Cr) levels in rats, which under normal physiological conditions is constant. By monitoring the level of total creatine(tCr) in vivo the potential untoward effects of seemingly inert vehicles can be measured. MRS was conducted on male SD rats under isoflurane using a 7T magnet. MRS signal was obtained from the hippocampus (64 cu mm voxel) by PRESS sequence with VAPOR water suppression (TE = 16.2 ms, TR = 3 s, NS = 512). After the baseline MRS scan, one of four different vehicles (2ml/kg) - saline (N = 4), or 1-2-97 (1% 1N HCl, 2% cremophor, 97% saline, N = 4), or DMA+PG (10% dimethyl acetamide (DMA), 45% propylene glycol (PG) and 45% saline, N = 9) or nanosuspension (0.025% sodium laurel sulfate and 2% poly-vinyl pyrrolidone in saline, N=4) was given subcutaneously, followed immediately by dynamic MRS scans. LCModel was used for tCr estimation. tCr (sum of phosphocreatine(PCr) and Cr) levels show a

statistically significant interaction (2 way repeated measures ANOVA) between time and excipients ($p<0.01$). 1-2-97 vehicle has no effect and closely matches the effect of saline on tCr. While the nanosuspension vehicle shows a trend toward elevated tCr over time the DMA+PG vehicle results in a rapid increase (20%) in tCr. Thus the DMA+PG vehicle by itself alters the stable tCr levels which will confound the interpretation of MRS studies when used as the vehicle for drug administration. PCr and Cr are coupled by the Lohmann reaction and are key in meeting the energy requirements and hence tCr is used as a marker of brain energy metabolism. Additionally since glia (and not neurons) can synthesize Cr, an alteration in Cr levels would point to a change in energy supply to the neurons thus serving[tCr levels] as a reliable marker of cellular integrity. In conclusion, one must be cautious when using MRS to assess changes in neurochemical profiles in the CNS. Thus, MRS is a sensitive tool for measuring acute changes due to excipients commonly assumed to be non-toxic.

PS 1929 DEVELOPMENT AND VALIDATION OF A PHARMACOKINETIC LIGAND BINDING-ASSAY FOR THE DETECTION OF A HUMANIZED THERAPEUTIC IN MULTIPLE SPECIES.

A. Neaga, Y. Boyle-Holmes, J. E. Coash, Y. Q. Xiao and L. D. Albee. *Department of Immunology, MPI Research, Mattawan, MI.* Sponsor: A. Faqi.

According to FDA regulatory guidelines, preclinical studies of monoclonal antibody (mAb) therapeutics should be conducted in relevant animal species. These studies should assess pharmacokinetic(PK) /pharmacodynamic (PD) characteristics of the therapeutics. The high homology and cross reactivity of many humanized therapeutics and endogenous proteins requires the development of highly selective PK ligand–binding assays which can systematically and discriminatively quantify circulating humanized therapeutics in the presence of endogenous proteins in the matrix of interest. As these studies can take place in multiple species with prolonged time for development of test article specific assays, it would be beneficial to develop a universal PK assay for mAb therapeutics. Achieving such an assay would decrease lead times necessary for starting studies and significantly impact costs. Therefore, we have developed a universal pre-clinical PK assay for the detection of human/humanized mAb's. This was validated using the Meso-Scale Discovery (MSD) electrochemiluminescence platform and demonstrates a broad dynamic detection range (i.e. 0.488 - 250 ng/mL for a mAb targeting an infectious disease) in matrix from multiple species. Preliminary evidence suggests that this method is adaptable to other platforms. The MSD-based methods were shown to be robust and reproducible, with inter and intra-assay coefficients of variation < 20%. Further, the assays had high accuracy with recoveries usually seen at much less than +/- 20% relative error. These studies illustrate the development of a validated assay with adaptability across multiple sources of matrix. Further adaptation of this method to other mAb has been rapid and demonstrates similar type of performance characteristics. Using this methodology we can develop pre-clinical PK assays to human/humanized mAb therapeutics without needing test article specific reagents, thus decreasing the time from assay development to core validation to less then 3 weeks.

PS 1930 A THOROUGH CHARACTERIZATION OF THE THE FREQUENCY RESPONSE OF VARIOUS *IN VIVO* BLOOD PRESSURE MEASUREMENT TECHNOLOGIES.

J. P. Kroehle, J. Grenwis and R. Sarazan. *DSI, St. Paul, MN.*

Since 1733 scientists have been measuring blood pressure in animals. Methods have evolved from acute experiments, including external strain gauge in 1945, to chronic applications using implantable strain gauge transducers with externalized wires in 1965 and fully implantable radiotelemetry devices in the late 1980's. With the advent of advanced pressure sensing solutions, scientists have an interest in using smaller animal models and measuring pressure signals, such as left ventricular pressure (LVP) that require greater levels of accuracy. To test the correctness of different monitoring systems available to measure systemic pressure and LVP in rodent and large animal models, several pressure sensing technologies were evaluated to compare performance. The first test evaluated frequency response (FR) of various pressure sensing systems used in rat models, including a dual pressure telemetry device (HD-S21, DSI), an intravascular pressure catheter (SPC-330A, Millar), an external pressure transducer attached to a 25cm catheter (P23XL, Statham) and a disposable external pressure transducer attached to a 25cm catheter (Deltran IV, Utah Medical). The same FR test was performed on large animal solutions, substituting the appropriate telemetry device (D70-PCTP, DSI), and an 80cm catheter for connection to the external transducers. Measuring the frequency response determines if the test system involved can adequately reproduce the pressure signal with appropriate morphology. Results confirmed that the disposable and Statham sensors provided FR values adequate for measuring systemic blood pressure signals, but not LVP signals. The implantable telemetry transmitters demonstrated FR values adequate for accurately measuring both systemic and LVP signals. A third test was performed to compare LVP signals collected from an HD-S21 and Millar catheter in an anesthetized rat. Data collected under control and challenge conditions demonstrated a high level of correlation between the two pressure signals and the corresponding peak value of the first derivative, dP/dtmax.

PS 1931 OPTIMIZATION OF INTRAVENOUS CYNOMOLGUS MONKEY INFUSION *VIA* VASCULAR ACCESS PORT.

A. M. Brooks and A. N. Alexander. *Department of Toxicology, Covance Inc., Madison, WI.*

Intermittent intravenous infusion is increasingly common in preclinical toxicology. Vascular access port catheters (VAPs) locked with heparin/saline allow animals to be disconnected from the infusion apparatus between doses, reducing opportunities for infection and improving quality of life. On this study, two commercially-available VAPs were evaluated with jugular or femoral catheter placement using ambulatory or tethered infusion to optimize infusion study design for cynomolgus monkeys. Infusion systems were assessed for external catheter breaks, needle dislodgements, leaks, occlusions, and disconnections; clinical signs, body weights, and clinical and anatomical pathology findings were used to evaluate tolerability. Eight groups of three monkeys received sterile lactated Ringer's solution (LRS) at 15 mL/kg/dose for 3 hours once daily for 14 days and then once weekly for 2 weeks. LRS was infused at 5 mL/hour during nondose intervals during the first 14 days to maintain catheter patency and simulate continuous infusion. For tethered infusion, a tether and jacket was employed to maintain connectivity to the pump. Needles bent with greater frequency with port type 1 while accessing the catheter via the port was more labor intensive and sterility more difficult to maintain with port type 2. Across both port types, femoral vein catheterization resulted in fewer non doseable days than did jugular vein. This difference was more pronounced for ambulatory infusion, particularly for port type 1; femoral vein placement in this model resulted in 86% less non-doseable (due to clinical signs and/or catheter issues) days than did jugular vein. No significant differences in body weight or clinical or anatomic pathology were observed. Transient minor clinical signs were less frequent in tethered animals than in ambulatory animals, likely due to the weight-bearing infusion jacket. Most combinations were acceptable for infusion in monkeys but femoral vein catheterization was preferable; the specific port/infusion system used for a given study should be selected based on the study design, dose duration, and study endpoints.

PS 1932 CONTROLLING FOR CROSS CONTAMINATION IN TOXICOLOGY STUDIES.

M. M. Peet, C. J. Dean, C. Diabo, E. St. Peter and S. E. Boley. *General Toxicology, MPI Research, Mattawan, MI.*

The presence of test compound in biological samples collected from control animals is a common occurrence in nonclinical safety studies regardless of the route, animal model, dosages, or study duration. Depending upon the overall significance and magnitude of contamination, the ability to accurately interpret the study findings may be compromised. Furthermore, investigations conducted to determine the cause of the contamination do not always allow for a definitive conclusion due to the numerous possible sources of contamination. As a result, the source of the test compound contamination is classified as "inadvertent cross contamination". A significant problem with these investigations is an inability to "see" the test compound, and, therefore, determine how such cross contamination could have occurred. The present study was conducted using Glo Germ™, a synthetic organic colorant visible under black light, to assess the potential for test compound contamination in standard toxicology study designs. The present study included single oral dose administration in rats, canine, nonhuman primates, and swine and dermal application once daily for 14 consecutive days in rats and swine. Oral gavage administration resulted in identification of Glo Germ™ predominantly on personal protective equipment (PPE) of laboratory staff and on the outside the bottles containing the test compound. Dermal application resulted in the presence of Glo Germ™ primarily on PPE of laboratory staff and equipment in the animal room. Traces of test compound were also observed on the outside of the blood collection tubes, although not after processing the collected blood to plasma. These findings indicated that contamination was not limited to one area during laboratory procedures. As a result, processes were reviewed and revised to limit the incidence of contamination. Conducting studies internally with Glo Germ™ may prove a useful tool for research facilities to assess which procedures are in need of modification to prevent cross contamination in toxicology studies.

PS 1933 PATHOLOGICAL LESIONS OF RODENTS LACKING HUMAN RELEVANCE IN RISK ASSESSMENT.

M. A. Mulla, R. Nirogi, S. Jana, V. Goyal and S. Pandey. *Toxicology, Suven Life Sciences Limited, Hyderabad, Andhra Pradesh, India.* Sponsor: V. Reddy.

Preclinical safety evaluation is performed to identify possible adverse effect in human. In routine toxicity studies, test substance related changes are observed in different toxicological parameters including histopathological findings. Test substance related histopathological changes may be species, strain or sex specific and may not have human relevance. Hence it is very important to know the relevance of test substance related histopathological changes observed in preclinical studies before considering them as adverse during risk assessment. The basic premise of our effort is to abridge and illustrate some pathological findings of rodents that are lacking human relevance in the context of interpretation, linking of observations and extrapolation of human health evaluation. Data were collected from scientific publications, workshops, seminars and conferences organized by industry and academia. To approach this perspective, we have accentuated here few paradigms like adrenal medullary proliferative lesions of rodents, chemically induced $\alpha 2\mu$ globulin nephropathy in aged male rats, peroxisome proliferations induced liver tumor, xenobiotics induced thyroid gland proliferation, Leydig cell adenoma in rodents, degenerative changes in nasal cavity that do not appear to have convincing evidence in humans or is often stated to be of low toxicological significance. Pathological lesions still having ambiguous human relevance need to be studied further. Hence, one of the key challenges is that pathological findings of rodents should be interpreted judiciously for extrapolation of human risk.

PS 1934 AN EVALUATION OF FACTORS FOR CLASSIFYING A CHEMICAL AS INDUCING CANCER IN EITHER RODENTS OR HUMANS *VIA* A MUTAGENIC MODE OF ACTION USING A WEIGHT OF EVIDENCE APPROACH.

D. A. Eastmond[1], S. V. Vulimiri[2] and B. Sonawane[2]. [1]*Environmental Toxicology Program, University of California Riverside, Riverside, CA and* [2]*National Center for Environmental Assessment, U.S. EPA, Washington, DC.*

The determination of whether or not a carcinogen acts through a mutagenic mode of action (mMOA) plays an important role in assessing risks associated with low dose exposure. However, the basis for determining that a carcinogen acts through a mMOA has not been established and is the subject of ongoing scientific debate. Towards this objective, we have evaluated factors that have played a role in human health risk assessments. A key component of a mMOA determination involves evaluating the outcome of various short-term genotoxicity tests and related factors and determining how much weight to place on the outcome of the information. In evaluating various risk determinations, we have developed a flexible and qualitative approach for the evaluation of both negative and positive test results. Consistent with common practice, more weight has generally been given to tests performed in vivo in mammalian systems than for those performed in vitro using mammalian cells or non-mammalian (yeast) or prokaryotic (bacteria) systems. Preference has also been accorded to results seen in the target organ over those seen in other non-target tissues. In addition to in vitro and in vivo genotoxicity test results, we have also proposed an approach for evaluating carcinogenesis-related information such as DNA reactivity, covalent DNA binding, structure-activity relationships, human biomonitoring results. By providing flexible procedures for evaluating and weighting test results, we believe that this approach will allow for scientific judgment as well as provide consistency in making future mMOA determinations. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

PS 1935 A WEIGHT-OF-EVIDENCE EVALUATION OF ASBESTOS EXPOSURE AND MESOTHELIOMA RISK AMONG ELECTRICIANS.

M. Peterson, L. A. Bailey, D. G. Dodge, J. E. Goodman and P. A. Valberg. *Gradient, Seattle, WA.*

Historically, some electricians may have been exposed to predominantly chrysotile asbestos fibers from a some electrical products and to a variety of asbestos fiber types if they worked proximate to other skilled craftsmen or renovation work that involved disturbing asbestos-containing insulation or other asbestos containing materials. Based on these potential exposure routes, we conducted a weight-of-evidence analysis to determine what levels and types of asbestos electricians could have been exposed to, and whether those exposures could have led to an increased risk of mesothelioma. Relevant exposure, toxicological, and epidemiological studies were evaluated based on a variety of factors, including their rigor, power, corroboration, relevance, and coherence within and among studies. Toxicological studies support an inflammatory mode-of-action for amphibole-induced mesothelioma, but the evidence for chrysotile causing mesothelioma is lacking. Based on our analysis of task-specific exposure studies as well as general occupational exposure studies, we found that potential for exposure to chrysotile asbestos encapsulated in electrical components was very low, while electricians' secondary exposure to amphibole asbestos originating from the activities of other tradesmen was relatively high. In addition, some, but not all, epidemiological studies reported elevated mesothelioma risks among electricians. Owing to a lack of exposure measurements, undocumented employment history, and small sample sizes, many of these studies could not determine whether asbestos was a causal factor, although several studies evaluated exposure in some fashion (including via lung biopsies and qualitative work history evaluations). Collectively, these studies support an association between mesothelioma risk and prior exposure to amphibole, but not chrysotile, asbestos. We conclude that the weight of evidence does not support an increased risk of mesothelioma among electricians, except for those in work environments with high levels of bystander amphibole exposure.

PS 1936 STATE-OF-THE-SCIENCE ASSESSMENT OF NON-FIBROUS AMPHIBOLE EXPOSURE: IS THERE A HEALTH HAZARD?

C. A. Williams, R. Adams, D. Podraza, C. Simmons, T. Rose and L. Dell. *Environ International, Tampa, FL.*

The distinction between fibrous (i.e., "asbestiform") amphiboles and non-fibrous ("non-asbestiform") amphiboles has important implications for assessing potential health risks associated with exposure to amphibole asbestos and amphibole-containing products. Exposure to fibrous amphibole asbestos can pose a health risk due to its ability to reside for long periods of time in the deep lung (i.e., biopersistence). In contrast, non-fibrous structures of amphibole minerals are usually cleared rapidly from the lung and therefore do not pose similar respiratory risk even at high doses. Most regulatory and public health agencies, as well as scientific bodies, agree that non-fibrous amphiboles possess reduced biologic (e.g., carcinogenic) activity. What is not agreed upon, however, are the specific characteristics that distinguish an amphibole asbestos fiber from non-fibrous structures and from fibrous structures lacking an asbestiform habit. Although non-fibrous asbestos minerals have been excluded historically from U.S. federal regulations, non-fibrous structures can be (and sometimes have been) counted as asbestos fibers on the basis of dimensional criteria specified in regulations. Given the potential to mischaracterize a non-fibrous and/or non-asbestiform structure as a "true" asbestos fiber, our objective was to assess whether exposure to non-fibrous amphiboles that may meet the dimensional criteria for asbestos fibers pose a health hazard similar to fibrous amphibole asbestos. We reviewed the toxicologic, epidemiologic, mineralogical, industrial hygiene, and analytical literature for non-fibrous amphiboles. We did not find evidence of demonstrable health effects from exposure to non-fibrous amphiboles that may, under certain assessment protocols, appear to be fibers. We did identify data gaps (industrial hygiene data for amphibole-exposed cohorts); inconsistencies (analytical laboratory methods/protocols used to count fibers); and sources of potential bias (from misclassification of exposure) in our review.

PS 1937 ARSENIC SPECIATION IN AIR: AN EVALUATION OF THE CURRENT STATE OF KNOWLEDGE AND RESEARCH NEEDS.

A. S. Lewis[1], K. R. Reid[1], M. C. Pollock[1] and S. Campleman[2]. [1]*Gradient, Cambridge, MA and* [2]*Electric Power Research Institute, Palo Alto, CA.*

Unlike arsenic (As) in drinking water, the potential health risks associated with exposure to As in ambient air are less studied. Risk assessment for As is typically based on total As, without regard to chemical speciation. We therefore conducted a comprehensive evaluation of currently available information on speciated As in air, including the type of species measured and their relative abundance, as well as an analysis of the limitations of current analytical methods and As risk assessment data gaps. The aim of this analysis is to identify critical data gaps, for which additional research will reduce uncertainty in quantification of exposure and potential risks to As in ambient air. Our analysis demonstrated that, despite limited comparability among studies due to differences in seasonal factors, study duration, sample collection methods, and analytical methods, research conducted to date is adequate to show that As in air is mainly in the inorganic form (AsIII and AsV reported average concentrations up to 7.4 ng/m3 and 10.4 ng/m3, respectively), with AsV being more prevalent than AsIII. Levels of the organic methylated As compounds are negligible (in the pg/m3 range). However, because of the variability in study methods and measurement methodology, we were unable to determine the variation in As composition as a function of source or PM fraction. We conclude that, in order to generate data more useful for As inhalation risk assessment, it is important to har-

monize sample preparation and analytical techniques, and a more robust documentation of QA/QC protocols is necessary to ensure accuracy, precision, representativeness, and comparability. Also, a consistent method of collection and analysis is needed. Accurate measurement of As in air is critical to providing a more robust understanding of As exposures and associated human health risks, particularly in the context of regulatory decision making.

PS 1938 THE ROLE OF IRON IN THE REDUCED BIOAVAILABILITY OF ARSENIC IN SOIL.

V. L. Mitchell[1], C. N. Alpers[2], N. T. Basta[3], T. Burlak[4], S. W. Casteel[5], R. L. Fears[1], A. L. Foster[6], C. S. Kim[7], P. A. Myers[1] and E. Petersen[8]. [1]*Toxic Substances Control, Cal EPA, Sacramento, CA,* [2]*U.S. Geological Survey, Sacramento, CA,* [3]*Ohio State University, Columbus, OH,* [4]*California State University, Sacramento, CA,* [5]*University of Missouri, Columbia, MO,* [6]*U.S. Geological Survey, Menlo Park, CA,* [7]*Chapman University, Orange, CA and* [8]*University of Utah, Salt Lake City, UT.*

Arsenic (As) is a naturally occurring element in soil and often the key chemical of concern at former mines in the California Mother Lode Region. Risk based screening levels are often below the naturally occurring background concentrations of As in soil. These screening levels assume 100% bioavailability, which can be greatly affected by the association of As with other elements. Iron(Fe)-bearing minerals are known to bind As and reduce its bioavailability; however the mechanism is not completely understood. The soils in the Mother Lode Region are relatively high in Fe, ranging from 2-10%. Total As in soils collected from the Empire Mine State Historic Park ranged from 16 to 9,700 mg/kg. Bulk soil samples were sieved to <250µm, the fraction that adheres to skin and is most likely to be ingested by humans. The mass of the <250µm fraction averaged 17.4% of the bulk sample (presieved <6mm). In vitro bioaccessible As (IVBA As), a surrogate for in vivo bioavailable As, in the <250µm fraction ranged from 1-44%. A strong linear relationship between IVBA As and Fe:As ratio (r^2=0.8244) was found. The spatial association between As and Fe was visualized utilizing QEMSCAN that provides quantitative chemical and mineralogical data at the 2.5µm pixel scale. Polished sections of mine waste rock analyzed by QEMSCAN demonstrated variable As content in hydrous Fe-oxide rims on weathered sulfide minerals. Further analysis, using a variety of synchrotron-based x-ray techniques, mapped the various oxidation states of As and Fe in these samples. Similar analysis of the <250µm fraction soil samples should provide useful information on the effect of Fe content and speciation on bioavailability of As in mining environments.

PS 1939 ARSENIC RISK ASSESSMENT FOLLOWING REMOVAL OF CONSTRAINING FACTORS—REFERENCE POPULATION AND MEDIAN EXPOSURE METRIC.

S. H. Lamm[1, 2], S. Robbins[1], J. Lu[3], R. Chen[4] and M. Feinleib[5]. [1]*Epidemiology, Consultants in Epidemiology and Occupational Health (CEOH), Washington, DC,* [2]*Health Policy and Management, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,* [3]*Mathematics, American University, Washington, DC,* [4]*Center for New Design in Learning and Scholarship, Georgetown University, Washington, DC and* [5]*Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.* Sponsor: J. Tsuji.

Background: The EPA Toxicological Review of Inorganic Arsenic (February 2010) and cancer risk assessment, based on bladder and lung cancer mortality data from the Blackfoot-disease (BFD) endemic area of southwest Taiwan, has demonstrated in its sensitivity analysis that its risk estimate is substantially affected both by its use of a reference population and its use of the median as the summary statistic for village arsenic exposure. Method: The BFD endemic area is a unique environment within Taiwan whose waters are characterized by high levels of both arsenic and humic substances and whose health data show high rates of bladder, lung, and skin cancers. The risk analysis attempts to assess the marginal increased bladder and lung cancer mortality attributable to increases in arsenic exposure in the < 150 ug/L range. Use of the median as the exposure metric confounds analysis as some villages show maximum arsenic levels that are greater than five times their median. Use of the maximum as the exposure metric eliminates that problem. Use of a reference population confounds the analysis with data from non-similar areas. We have replicated the analyses without these two constraints to the model. Results: The carcinogenic risk slope factor is significantly positive over the range of 200-800(+) ug/L. A marked inflection occurs in the range of 100-200 ug/L (depending on model parameters). Non-positive slopes occur at levels below 100 ug/L. Conclusion: The assessment of the carcinogenic risk of ingested inorganic arsenic at low exposures (< 150 ug/L) should be based on data from low exposure (< 150 ug/L) villages. As such, the models do not demonstrate a positive slope in that range.

PS 1940 INTERPRETING NHANES DATA ON ARSENIC LEVELS IN URINE USING BIOMONITORING EQUIVALENTS.

C. R. Kirman[1], S. M. Hays[2], L. L. Aylward[3] and R. Schoeny[4]. [1]*Summit Toxicology LLP, Orange, OH,* [2]*Summit Toxicology LLP, Lyons, Co.,* [3]*Summit Toxicology LLP, Falls Church, VA and* [4]*U.S. EPA, Washington, DC.*

The National Health and Nutrition Examination Survey (NHANES) reported data on concentrations of speciated and total arsenic in urine for a sample of the general population. Adverse effects associated with arsenic exposures are thought to be from exposure to inorganic species, which are excreted in urine in a variety of forms: inorganic arsenic (iAs), monomethyl arsenic (MMA), and dimethyl arsenic (DMA). A Biomonitoring Equivalent (BE) has been derived for inorganic-derived arsenic species in urine, which provides an estimate of the concentration of iAs, MMA, DMA and their sums in urine that are consistent with reference values, such as the US EPA reference dose (BERfD). The BE can be used to interpret the arsenic biomonitoring data in the context of the RfD. The sum of all inorganic derived arsenic species (iAs, MMA and DMA) from the NHANES 2007-8 collection period exceed the BERfD at around the 50th percentile, although the proportion exceeding the BERfD was affected by the treatment of non-detects. A significant proportion of the summed urine concentrations is due to DMA, which is assumed to result predominantly from exposure to inorganic arsenic exposure. However, the NHANES biomonitoring data indicate a correlation between DMA and seafood-derived organic arsenic species (arsenocholine and arsenobetaine), suggesting that DMA in urine may also arise from seafood consumption. When DMA is omitted from summed inorganic arsenic species (sum of iAs and MMA), 90% of the NHANES biomonitoring data are below the BERfD. The BE associated with the 1E-4 cancer risk is a factor of ten lower than the BERfD and is below the limit of detection for the NHANES analyses. This presentation will explore the various advantages and disadvantages of analyzing the various arsenic species in urine in a risk assessment context and make recommendations for future efforts. The opinions are those of the authors and do not necessarily reflect policies of U.S. EPA.

PS 1941 A REVIEW OF THE POTENTIAL ASSOCIATION BETWEEN CHILDHOOD LEUKEMIA AND BENZENE.

D. Pyatt[1, 2, 3] and S. Hays[1, 2]. [1]*Summit Toxicology, Superior, Co.,* [2]*School of Public Health, University of Colorado, Denver, CO and* [3]*MTEHS Program, University of Colorado, Denver, CO.*

Chronic exposure to high concentrations of benzene is an established cause of acute myeloid leukemia (AML) in occupationally exposed workers. Based on this association, it is not unreasonable to assume that children could also get AML if they were exposed to comparable levels of benzene. Fortunately, reports of such exposures and subsequent AML development in children are non-existent. However, the question of whether children can develop leukemia at far lower, environmental levels of benzene remains. The existing scientific evidence relevant to this question has been comprehensively evaluated and is presented herein. We evaluated all published epidemiology studies (over 100) that presented data relevant to this issue. Most studies included one or more of the following exposure pathways for benzene: 1) direct exposure to the child (via a variety of sources); 2) maternal exposures during pregnancy or while breastfeeding; and 3) exposure to either parent before conception. This analysis also included cigarette smoking, as second hand smoke is one of the main routes of benzene exposure in children. These pathways are not mutually exclusive and frequently occur together. Unfortunately, few studies contained quantified exposure information and most have no meaningful exposure estimates at all. The use of various surrogates for exposure such as proximity to gas stations or traffic density makes interpretation difficult. Parental exposures to benzene or other chemicals in the workplace and its potential effect in the offspring have also been assessed.

While positive findings have been reported, a comprehensive 'weight of the evidence' analysis of this literature does not indicate that exposure to environmental levels of benzene is related to an increased risk of childhood leukemia. Our understanding of this important issue would be strengthened by additional studies that accurately characterize exposures as well as differentiate between the various forms of leukemias observed in children.

PS 1942 USE OF *IN VITRO* BIOACCESSIBILITY ANALYSIS, HUMAN HEALTH RISK ASSESSMENT, AND URINARY ARSENIC MEASUREMENTS TO ASSESS EXPOSURE AND RISKS OF CHILDREN IN A NORTHERN CANADIAN SMELTER COMMUNITY.

E. Sigal, A. M. Safruk and C. M. Bacigalupo. *Intrinsik, Mississauga, ON, Canada.*

A mine and base metal smelting complex was established in Flon Flon, Manitoba, Canada in the 1930's, producing copper, cadmium, and zinc metals. Emissions from the smelter have resulted in elevated concentrations of a number of metals

and metalloids in the surrounding environment. As part of the larger Flin Flon Soils Study, a human health risk assessment (HHRA) was conducted between July 2007 and May 2010 to assess exposure and risks associated with a number of metals and metalloids, including arsenic. An in vitro bioaccessibility study was completed on residential soils to adjust predicted exposure estimates related to incidental soil ingestion. The HHRA indicated that arsenic was present at concentrations in the local environment that warranted further attention. A urinary arsenic study was therefore undertaken in the fall of 2009 and involved the collection urine samples from 379 children (2 to 14 years of age) in the affected community. Predicted inorganic arsenic exposure estimates (μg/kg bw/day) were converted to urinary inorganic arsenic concentrations (μg/L) to provide a meaningful comparison to measured data. To determine whether or not children from the Flin Flon area have significantly different urinary inorganic arsenic levels from those children who do not reside near a base metal smelting complex, measured data from this study were compared to measured urinary inorganic arsenic data taken from children living in non-exposed communities. The decision to conduct a urinary arsenic study was predicated on the results of the HHRA. The risk estimates in the HHRA were characterized using a cancer slope factor recommended for use by the U.S. EPA which was derived, in part, using human epidemiology data from Taiwanese drinking water studies during the 1960's and 70's. Despite the use of a site-specific bioaccessibility study, the results of this study highlight the need for a weight-of-evidence evaluation when characterizing health risk associated with exposure to inorganic arsenic.

PS 1943 BLOOD AND URINARY ELIMINATION KINETICS OF BISPHENOL A IN HUMANS FOLLOWING DIETARY EXPOSURE.

J. G. Teeguarden[1], R. Gunawan[1] and A. Calafat[2]. [1]*Biological Monitoring and Modeling, Pacific Northwest National Laboratory, Richland, WA and* [2]*Organic Analytical Toxicology Branch, National Center for Environmental Health, Centers for Disease Control, Atlanta, GA.*

Bisphenol A (BPA) is a now controversial monomer used in the manufacture of polycarbonate plastics and found in trace quantities in some consumer products and food containers. Unconjugated BPA is a low affinity ligand of the estrogen receptor(s) comparable to other natural estrogen receptor ligands in the food supply such as genestein and daidzein. Exposure to BPA has been widely estimated from urine spot samples and various single measures in blood and tissues. However, neither the pharmacokinetics of BPA nor the relationship between blood concentrations and amounts measured in urine for dietary exposures in humans have been described. Blood and urine samples were collected hourly from twenty healthy adult volunteers over a 24 hour period, and analyzed for total BPA. There was considerable inter- and intra-meal variability in the amount of BPA excreted in urine, as well as intra- and inter-person variability in elimination. The average total daily exposure to BPA based on the 24 hour sum of BPA eliminated was 20.96 μg (0.125 μg/kg) with a range of 3.29 to 73.29 μg. Only volunteers eliminating greater than approximately 12 μg of BPA in urine for any between-meal interval had consistently quantifiable levels (detection limit 0.3 ng/ml) of BPA in serum over the same interval. The blood kinetics demonstrated rapid uptake, and area under the curve to dose and peak to dose ratios very similar to those reported for a much higher, controlled dose of 5000 μg, showing wide linearity in the kinetics of BPA across the low-exposure range relevant to human exposures. The study also demonstrates that the episodic nature of oral exposure to BPA and rapid blood and urine kinetics confound translation of spot sample BPA concentration into human exposure estimates if kinetics are not accounted for. An approved IRB protocol was followed. Supported by U.S. EPA STAR GRANT R83386701.

PS 1944 WEIGHT-OF-EVIDENCE EVALUATION OF FORMALDEHYDE EXPOSURE AND LEUKEMIA RISK.

J. E. Goodman, D. B. Mayfield, L. A. Bailey and L. R. Rhomberg. *Gradient, Cambridge, MA.*

We conducted a weight-of-evidence evaluation to assess whether epidemiology data support an association between formaldehyde exposure and leukemia. This evaluation considered studies of individual lymphohematopoietic cancers as well as several groupings of these cancer types. We considered how the definition and classification of lymphohematopoietic cancers may have affected results of epidemiology studies and assessed whether evaluations of other cancer types or several cancer types combined have implications regarding leukemia risks from formaldehyde exposure. We also evaluated whether results were consistent for different types of exposure metrics (e.g., peak exposure, number of peak exposures ≥ 4.0 ppm, cumulative exposure) and whether results were dependent on the robustness of exposure measurements, with a particular focus on the NCI industrial worker and embalmer cohorts. We determined whether there were consistent exposure-response associations and assessed potential statistical limitations among studies. We found that there is no lymphohematopoietic cancer or group of lymphohematopoietic cancers for which associations with formaldehyde were found consistently within or among studies. Although some statistically significant associations were reported, these were outnumbered by null findings using related exposure metrics, and there were no consistent exposure-response relationships observed. Limitations in exposure and cancer outcome assessments and statistical analyses also likely affected calculations of risk. If formaldehyde were truly a causal factor for leukemia, consistent observations of effect should have been observed, with increased risks found with increased levels of exposure. Because this is not the case, it is most likely that any observed effects were a result of confounders, limitations in statistical methods (e.g., multiple comparisons), disease misclassification, and/or exposure misclassification/measurement error.

PS 1945 WEIGHT-OF-EVIDENCE EVALUATION OF NON-CANCER ANIMAL AND HUMAN STUDY OUTCOMES AFTER FORMALDEHYDE EXPOSURE.

S. L. Makris, T. F. Bateson, D. DeVoney, S. Y. Euling, B. S. Glenn and K. C. Raffaele. *ORD, NCEA, U.S. EPA, Washington, DC.*

Formaldehyde (FA) is a reactive gas found ubiquitously in ambient and indoor air, sometimes at relatively high concentrations (e.g., in mobile homes, laboratories). EPA's IRIS Program conducted an evaluation of the animal toxicology and epidemiology literature for FA-associated effects and released a draft document for public review in 2010 summarizing the weight of the evidence. This evaluation demonstrates respiratory effects, specifically pulmonary function decrements, and exacerbation of asthma in children. Importantly, multiple types of exposure-related systemic effects were found, increased allergic sensitization, alterations in nervous system function and behavioral perturbations (such as learning deficits and neurodevelopmental changes), effects on development (fetal loss, structural alterations, growth retardation, and delays in functional ontogeny), and evidence of both male and female reproductive toxicity (e.g., testicular and sperm effects in males and early pregnancy disruptions in females). Consistency in the types of reproductive effects was observed across animal toxicology and human epidemiological studies. For example, a study of women employed in the wood-processing industry found a significant exposure-response association between formaldehyde exposure and increased risk of spontaneous abortion, decreased fertility, and increased time to pregnancy; in animal studies early fetal death was observed after formaldehyde exposures. Overall, the toxicological and epidemiological dataset support public health concerns regarding the responses of sensitive populations to FA exposure. In spite of the overall strength of the evidence that supported the characterization of the non-cancer hazard associated with FA exposure, the toxicological database has a number of data gaps, and research needs have been identified. Disclaimer: This abstract does not necessarily reflect the views or policies of the U.S. EPA.

PS 1946 EFFECT OF FORMALDEHYDE EXPOSURE ON UPPER RESPIRATORY TRACT CANCERS: WEIGHT OF EVIDENCE.

T. F. Bateson, J. Jinot, R. P. Subramanian, D. DeVoney, S. V. Vulimiri and J. Whalan. *ORD/NCEA, U.S. EPA, Washington, DC.*

EPA systematically evaluated the epidemiologic literature on the relationship between formaldehyde exposures and upper respiratory tract (URT) cancers in humans. EPA's draft IRIS assessment of formaldehyde concludes that the weight of the evidence supports a causal association between formaldehyde exposure and rare URT cancers – especially nasopharyngeal cancer (NPC) and sinonasal cancer. The Hauptmann et al (2004) analysis of the large NCI cohort study of 10 worksites reported a doubling of NPC mortality risk (SMR=2.1, 95%CI 1.05-4.21) and demonstrated exposure-response relationships with cumulative exposure to formaldehyde (p-trend=0.025) and peak exposure to formaldehyde (p-trend<0.001) using internal controls. A robust collection of data from case-control studies also identified exposure-response relationships adding weight to the causal evidence of an association. Luce et al (2002) standardized data from 12 separate studies and pooled them in a single analysis which provided the strongest evidence that formaldehyde is associated with sinonasal cancer. Taken together with the NPC findings in the neighboring tissue of the URT, this is evidence of a causal association of sinonasal cancer with formaldehyde exposure. The evidence for a specific effect of formaldehyde exposure on the risks of buccal/pharyngeal, oral, oropharyngeal, hypopharyngeal, and laryngeal cancers is slight when examining each of the other rare URT endpoints individually. However, the large NCI study showed a statistically significant increased risk for URT cancers as a group and for cancers of the buccal cavity. Further evidence includes a nearly 4-fold increased risk of hypopharyngeal cancer (Laforest et al, 2006) and the demonstration of an exposure-response relationship for duration of formaldehyde exposure and risk of laryngeal cancer (Shangina et al, 2006). Taken together with the causal evidence for

NPC and sinonasal cancer in neighboring tissues of the URT and other sites of first contact with inhaled exposure, these collective results support a broader pattern of carcinogenicity within the URT.

PS 1947 EVIDENCE-BASED ANALYSIS DEFINING A FORMALDEHYDE VAPOR CONCENTRATION NOEL FOR IRRITANT RESPONSES IN HUMANS.

J. K. Britt[1], N. Halmes[2], B. Kerger[3] and R. C. James[1]. *[1]TERRA, Inc., Tallahassee, FL, [2]Halmes & Associates, Centennial, CO and [3]HSRI, Inc., Tallahassee, FL.*

The available scientific literature contains a broad range of human studies suggesting a range of levels at which formaldehyde may begin to induce eye or upper respiratory irritation. This study attempts to resolve the current uncertainty by an analysis derived via methods developed for reaching an evidence-based medical/toxicological conclusion. A literature search was conducted to identify all potentially relevant studies. The literature has shown that subjective symptoms of irritation are affected by mood (negative affect), the individual's pre-conceived or naïve interpretation of the possible hazard associated with an odor, and that eye irritation is the most sensitive endpoint. Studies were rated and ranked according to preselected experimental design components that included these considerations. Studies that inadequately addressed certain rating components were eliminated altogether. Not surprisingly, experimental studies with carefully controlled exposures, using physiologic responses to derive objective measurements, and carefully controlled for confounding ranked and rated the highest and provided the best available evidence. The present evidence-based analysis identified a NOEL for eye irritation of 0.6 ppm formaldehyde and a LOEL of 1.0 ppm with a unidentified threshold falling somewhere between these two levels. If subjective symptom measurements, which comprised the majority of the studies, were considered separately, and a well tolerated subjective interpretation of the exposure was the desired measurement outcome then a somewhat higher level near 1.0 ppm would represent the likely No Observable Adverse Effect Level.

PS 1948 WEIGHT-OF-EVIDENCE EVALUATION OF A PLAUSIBLE MODE OF ACTION FOR LEUKEMOGENESIS FROM INHALATION EXPOSURE TO FORMALDEHYDE.

L. Bailey, A. K. Hamade and L. R. Rhomberg. *Gradient, Cambridge, MA.*

We conducted a weight-of-evidence evaluation to assess the plausibility of three modes of action that have been proposed for formaldehyde (FA) leukemogenesis: 1) FA directly targeting bone marrow; 2) FA targeting nasal stem cells in the nasal-associated lymphoid tissue (NALT); or 3) FA targeting circulating hematopoietic stem cells (HSC), with later incorporation of nasal stem cells or HSCs into bone marrow. We determined whether the concentrations of FA that would be necessary to exceed endogenous levels in tissue are relevant to typical human exposures, and whether these concentrations would cause irritation. We determined whether the available evidence is consistent with FA-induced genotoxicity directly in the bone marrow, NALT, or circulating HSCs, and whether the types of DNA damage observed in FA genotoxicity studies are consistent with typical DNA damage associated with leukemias. And finally, we examined the likelihood that damaged circulating stem cells home back to healthy bone marrow to induce leukemia. We found that under typical human exposure concentrations, FA's reactivity precludes its movement past the immediate respiratory epithelia following inhalation, precluding its ability to cause DNA damage in the NALT or circulating HSCs. We found that higher FA exposures (necessary to exceed endogenous levels) would likely be associated with a high degree of irritation. We found these results to be consistent with our further observation that cytogenetic effects typically reported in support of potential distant-site FA toxicity were inconsistent, potentially confounded by other exposures, not specific to leukemias, and typically reported in human peripheral blood lymphocytes which should not be presumed to equate with circulating HSCs. Finally, we found that there are many questions regarding HSC homing to bone marrow under homeostatic conditions. We conclude that the current weight of evidence, considering metabolism, distribution, genotoxicity, and HSC homing provides little support for the proposed modes of action for FA leukemogenesis.

PS 1949 DOES FORMALDEHYDE CAUSE HEMATOTOXICITY? A WEIGHT-OF-EVIDENCE EVALUATION OF HEMATOTOXICITY STUDIES IN HUMANS AND ANIMALS IN THE CONTEXT OF LEUKEMOGENICITY.

A. Hamade, L. A. Bailey and L. R. Rhomberg. *Toxicology, Gradient, Cambridge, MA.*

We conducted a weight-of-evidence evaluation to assess the plausibility of recently proposed hypotheses that suggested potential hematotoxic and leukemogenic properties for formaldehyde. First, we evaluated studies in humans that assessed hematology outcomes in subjects occupationally exposed to formaldehyde. We found no indication from these studies that formaldehyde is hematotoxic. Some studies have shown some evidence of changes in hematology in occupationally exposed populations; however, not all details of these studies are available, the studies provide mixed evidence of effects, and some studies report statistically significant outcomes even at concentrations that may be exceeded in some public spaces. Even when some studies show evidence of hematology changes, the changes are not clinically significant and may be explained by mechanisms independent of bone marrow toxicity. Second, we evaluated hematotoxicity studies in animals. We found generally no convincing evidence that formaldehyde, even at very high exposure concentrations, could cause hematotoxicity. Moreover, our evaluation revealed that animal studies do not show evidence of leukemia induction by formaldehyde exposure, a conclusion that seriously weakens the argument for its induction in humans. Only one study reports formaldehyde-associated leukemia in animals, and this study has serious faults that discredit its outcomes. Third, we examined the hematotoxic effects of established leukemogens, such as benzene and cyclophosphamide, and found that these agents invariably induce bone marrow toxicity and hematotoxicity, suggesting that if formaldehyde causes leukemia in humans, it is likely independent of hematotoxicity, which would be inconsistent with any mechanism currently known for leukemogenesis. In conclusion, we did not find convincing evidence that formaldehyde is hematotoxic in animals or humans.

PS 1950 IS MTBE GENOTOXIC OR MUTAGENIC?

M. Seeley, L. A. Beyer and B. D. Beck. *Gradient Corporation, Cambridge, MA.*

Due to prior use as a fuel oxygenate, methyl-tert-butyl-ether (MTBE) has been found in groundwater, where it presents a concern for potential human health risks, primarily cancer. There is no evidence that MTBE causes cancer in humans. Increased cancer incidence has been observed in several animal cancer bioassays, but only at exposure levels several orders of magnitude greater than potential exposures to MTBE in groundwater. The mode-of-action and nature of the dose-response for MTBE carcinogenesis is currently a matter of scientific debate, with important ramifications for establishing permissible concentrations of MTBE in groundwater. Key to that debate is whether MTBE is genotoxic or mutagenic, in that evidence of mutagenicity would be potentially supportive of a no threshold dose-response model. We therefore conducted a weight-of-evidence analysis of MTBE genotoxicity assays, including those from both published studies, and unpublished studies discussed in regulatory documents. We identified 13 in vivo and 28 in vitro assays, including mutagenicity assays (24) as well as "indicator" assays (17) (i.e. tests, such as sister chromatid exchange assays, that reflect genotoxicity, but do not confirm mutagenicity). Importantly, none of the in vivo, and only one in vitro mutagenicity assay yielded a positive result with MTBE; subsequent evaluations indicate this result was due to exogenous formation of formaldehyde, specific to the conditions of the in vitro assay, rather than intrinsic mutagenicity of MTBE. Based on a critical analysis of the 12 indicator assays that provided some evidence of DNA damage under certain conditions, we identified important limitations for these assays, including minimal nature of the response, lack of a clear dose-response, problems with study design, methodological issues, as well as differences between in vitro and in vivo metabolism of MTBE. We conclude that the weight of the evidence indicates that MTBE is unlikely to be genotoxic or mutagenic. Thus margins-of-exposure analyses are more appropriate than linear no-threshold models for use in quantification of MTBE cancer risks

PS 1951 METHYL TERTIARY BUTYL ETHER (MTBE): TWO-YEAR DRINKING WATER STUDY IN WISTAR RATS.

E. Bermudez[1], D. K. Layko[1], G. A. Willson[2] and D. E. Dodd[1]. *[1]The Hamner Institutes for Health Sciences, Research Triangle Park, NC and [2]Experimental Pathology Laboratories, Inc., Research Triangle Park, NC .*

To improve the human health risk assessment of MTBE in drinking water, male and female Wistar rats (50/sex/group) were exposed to drinking water, ad libitum, containing various concentrations of MTBE, 7 d/wk, for 2 yr. MTBE drinking water concentrations were 0 (control), 0.5, 3, and 7.5 mg/mL for male rats, and 0, 0.5, 3, and 15 mg/mL for female rats. Endpoints included clinical observations, body weights, food and water consumption, gross necropsy, organ weights, and tissue histopathology. Body weights and food consumption were unaffected by exposure, but water consumption was reduced in all MTBE-exposed groups (e.g., 77, 71 and 69% of the control for the 0.5, 3 and 7.5 mg/mL male groups, respectively). Statistical analyses of animal survival indicated no differences in survival between control and MTBE groups. Following 24 months of MTBE exposure, statistically significant increases in left and right kidney weights were observed in male and female rats of all MTBE exposure groups. Microscopically, a statistically significant increase in the incidence and severity of chronic progressive nephropathy (CPN), a

rodent-specific disease, was observed in males (7.5 mg/mL) and females (15 mg/mL), with a greater incidence and severity in males. In male rats, a statistically significant trend of increasing CPN severity with increasing MTBE concentration was observed. Neoplasms of noteworthy incidence were limited to the brain. Astrocytomas were identified in study animals (9/460) with incidences in male rats, continuously exposed to MTBE, of 1/50, 1/50, 1/50, and 4/50 for the 0, 0.5, 3, and 7.5 mg/mL groups, respectively. Statistical analysis indicated a marginally significant (p=0.037) trend in male rats, however this trend was not considered biologically significant because the observed incidences of astrocytoma fell within historical control ranges published in the literature for Wistar rats and the brain has not been identified as a target organ in previous MTBE carcinogenicity studies.

PS 1952 A COMPARATIVE RISK EVALUATION OF MTBE AND OTHER COMPOUNDS (INCLUDING NATURALLY OCCURRING COMPOUNDS) IN DRINKING WATER IN NEW HAMPSHIRE.

L. A. Beyer, R. L. Mattuck, S. Thakali and B. D. Beck. *Gradient, Cambridge, MA.*

We conducted a comparative cancer risk assessment for several constituents in NH drinking water. The overall aim was to present a relative ranking of risks, not to evaluate whether permissible limits were exceeded. Using standard risk assessment methodology, we calculated relative cancer risks from exposure to ten VOCs (including MTBE) detected in NH public water supplies, as well as arsenic, radium-226, radium-228, and radon. We evaluated exposures to an adult resident via ingestion of water, dermal contact with water while showering, and inhalation of volatilized compounds in the home. For arsenic, we evaluated only the ingestion and dermal pathways; for the radionuclides, we evaluated only the ingestion and inhalation pathways. This comparative risk evaluation is similar to one conducted by Williams et al. (2002, 2004) for water supplies in California. From this analysis, we concluded that the upper bound cancer risk from exposure to MTBE in drinking water is lower than the risks from nine other VOCs detected in NH drinking water supplies, and several thousand times lower than the risks from exposure to naturally occurring constituents including arsenic, radium isotopes, and radon. Even with the risk weighted by detection frequency, MTBE exhibits the third lowest risk of the VOCs and exhibits a relative risk two orders of magnitude lower than the relative risk for the disinfection byproducts (bromodichloromethane, chloroform, and dibromochloromethane).

PS 1953 SUB-CHRONIC NAPHTHALENE INHALATION CAUSES A DECREASE IN *P53* CODON 271 CAT MUTANT FRACTION IN THE NASAL RESPIRATORY EPITHELIUM OF MALE RATS.

F. Meng[1], Y. Wang[1], M. B. Myers[1], B. A. Wong[2], E. A. Gross[2], H. J. Clewell[3], D. E. Dodd[2] and B. L. Parsons[1]. *[1]Division of Genetic and Molecular Toxicology, National Center for Toxicological Research/U.S. FDA, Jefferson, AR, [2]Division of Toxicology and Preclinical Studies, The Hamner Institutes for Health Sciences, Research Triangle Park, NC and [3]Center for Human Health Assessment, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

This study sought to gain mechanistic insight into naphthalene-induced nasal carcinogenesis through dose response analysis of a tumor-relevant endpoint. Levels of *p53* codon 271 CGT to CAT mutation were measured in rat nasal respiratory and olfactory epithelium following whole-body exposure to naphthalene (NA) by allele-specific competitive blocker-PCR. Male and female, 6-7 week-old F344 rats were exposed to 0, 0.1, 1.0, 10, and 30 ppm NA vapor for 13 weeks (6 h/day, 5 days/week), using 5 rats/group. For control groups, the geometric mean *p53* mutant fraction (MF) levels in nasal epithelium ranged between 2.05 X 10^{-5} and 3.05 X 10^{-5}. No significant dose-related changes in *p53* MF were observed in the olfactory or respiratory epithelia of female rats. However, statistically significant treatment-related differences were observed in male respiratory and olfactory epithelium, with the *p53* MF in the respiratory epithelium of male rats exposed to 30 ppm NA significantly ($p<0.05$) lower than that of controls. Further, a significant ($p<0.05$) trend of decreasing *p53* MF with increasing dose was observed in the male respiratory epithelium. Of the tissue types analyzed, respiratory epithelium is the most sensitive to the cytotoxic effects of NA, suggesting cytotoxicity may be responsible for the loss of *p53* mutation. Because ACB-PCR has been used successfully to detect the effects of known mutagenic carcinogens, the absence of any significant increase in *p53* MF associated with NA exposure adds to the weight of evidence that NA does not operate through a directly mutagenic mode of action. This study was supported in part by the Naphthalene Research Committee.

PS 1954 NASAL EPITHELIAL LESIONS IN F344 RATS FOLLOWING A 90-DAY INHALATION EXPOSURE TO NAPHTHALENE.

D. E. Dodd[1], B. A. Wong[1], E. A. Gross[1] and R. A. Miller[2]. *[1]The Hamner Institutes for Health Sciences, Research Triangle Park, NC and [2]Experimental Pathology Laboratories, Inc., Research Triangle Park, NC .*

Naphthalene (NA) was shown to be carcinogenic, causing respiratory epithelial adenoma in the nasal cavity of male F344 rats and olfactory epithelial neuroblastoma in female F344 rats at exposure concentrations of 10 to 60 ppm in a 2-yr inhalation study. To explore the exposure-response relationship and threshold for nasal epithelial effects in F344 rats, a 90-day (6 h/d, 5 d/wk) inhalation study was conducted at 0, 0.1, 1, 10, and 30 ppm NA vapor. Group size for nasal cavity histopathology was 10/sex with an additional 10/sex evaluated for recovery 4 wk post-exposure. NA exposure concentrations were measured by GC/MS, and aerosol testing verified that solid NA particles were not present. There were no NA exposure-related clinical observations and mild decreases in body weight (<10%) and food/water consumption were observed primarily in the 30 ppm exposed rats. Thymic weights were decreased (<17%) in males exposed to 10 or 30 ppm. Decreased weights of thymus (<16%) and liver (<11%) were observed in females of all NA groups. Body and organ weight effects diminished following recovery. Rat heads were cross-sectioned at six levels for microscopic examination (H&E). There were no nasal cavity lesions related to NA exposure in rats of the 0.1 ppm group. Minimal hyperplasia was observed in the transitional/respiratory epithelium of rats exposed to 1 ppm. Mild hyperplasia and minimal squamous metaplasia were observed in the respiratory epithelium of rats exposed to 10 or 30 ppm. Lesions in the olfactory epithelium were observed only in rats of the 10 or 30 ppm groups and consisted of degeneration, necrosis, areas of re-epithelialization and basal cell hyperplasia. There was remarkable recovery of effects after 4 wk, but residual olfactory epithelial degeneration and basal cell hyperplasia were still evident. There were no discernible differences between male and female rats in either incidence or severity of NA-induced nasal cavity lesions. Work sponsored by the Naphthalene Research Committee.

PS 1955 KEY MODE-OF-ACTION ISSUES AND DATA GAPS FOR NAPHTHALENE –INDUCED CARCINOGENICITY.

C. Keshava[1], P. McClure[2], J. Stickney[2] and L. Flowers[1]. *[1]NCEA, ORD, U.S. EPA, Washington, DC and [2]SRC, Inc., Syracuse, NY.*

Mode of action (MOA) analysis is a key component of hazard characterization and dose-response assessment. The MOA of naphthalene-induced carcinogenicity has been the subject of scientific discussion, particularly with respect to the human relevance of the observed tumors and the shape of the dose-response curve at low dose. U.S. EPA's Integrated Risk Information System (IRIS) program is conducting a reassessment of the health effects associated with naphthalene exposure, including cancer and noncancer effects. Animal carcinogenicity data indicate clear evidence of nasal tumors in rats (both sexes) but not in mice, some evidence of lung tumors in female mice and no evidence of lung tumors in male mice. Numerous studies on the MOA of naphthalene-induced carcinogenicity are available, however, most are either not conducted in the target tissues or at appropriate doses. Proposed key MOA events leading to naphthalene-induced nasal tumors in rats and lung tumors in mice include metabolic activation, exceedance of detoxification capacity, covalent modification of key proteins, site-specific DNA damage and mutagenicity, and cytotoxicity, but two important questions are unanswered. Why do only rats develop nasal tumors, but both rats and mice develop nonneoplastic nasal lesions? Why do mice develop lung lesions and tumors, but not rats? Data gaps for further understanding of the MOA of naphthalene-induced carcinogenicity include: examining metabolism of naphthalene downstream from the initial epoxide formation; identifying toxicokinetic differences between primates and rodents; examining possible genotoxic events in target tissues; and availability of subchronic or chronic studies at concentrations less than 10 ppm. This information could help inform the evaluation of dose-response at low doses and the temporal relationships between nasal effects and tumor formation in rats, and lung effects and tumor formation in mice. Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

PS 1956 OVERVIEW OF EPIDEMIOLOGY DATA FOR INDUSTRIES WITH NAPHTHALENE-CONTAINING STREAMS.

R. J. Lewis. *ExxonMobil Biomedical Science Inc., Annandale, NJ.* Sponsor: M. Bird.

Naphthalene is a natural constituent of petroleum and is a product of incomplete combustion of biomass and petroleum products. The National Toxicology Program (NTP) conducted two bioassays in mice and rats exposed to naphthalene, which

showed incidences of lung and nasal cancer, respectively. These bioassay findings have raised questions of human relevance and cancer risk. To address this question, studies of industries with naphthalene-containing streams having the most extensive epidemiology data available were reviewed; this included studies involving petroleum refining, asphalt (paving and roofing), and creosote production, as well as jet fuel handlers. The review focused on respiratory cancer (nasal and lung cancer) as these are the tumors types seen in the animal studies. The review identified no epidemiology studies of workers exposed only to naphthalene. There are limited case reports of laryngeal and colorectal cancer related to naphthalene exposure, but expert bodies have concluded these data are inadequate for evaluating human cancer risk. Notably, there are no case reports of nasal tumors for workers in industries with naphthalene-containing streams. The lack of case reports for a rare tumor like nasal cancer is important given that case reports (not formal cohort studies) have historically identified several occupational carcinogens that cause rare tumor types (e.g., vinyl chloride and angiosarcoma of the liver). When combined with results of Magee et al. (2010) indicating that the cancer potency estimates based on the rat NTP nasal tumor findings significantly overestimate the predicted total number of nasal tumors in the U.S. population, the relevance of rat nasal tumors to humans is questionable. For lung cancer, the lack of case reports is not informative due to the high background rate of this tumor and its relation to smoking. Further interpretation of the human data for lung cancer is complicated by limitations of cohort and/or case-control studies (e.g., low exposure, confounding by smoking), although no occupationally-related risks were identified in the industries evaluated

PS 1957 NAPHTHALENE RESEARCH: THE RELEVANCE OF TUMORS IN RODENTS TO HUMAN RISK ASSESSMENT.

A. LeHuray[1] and K. Wise[2]. [1]*Naphthalene Council, Alexandria, VA and* [2]*American Petroleum Institute, Washington, DC.*

Industry associations and individual companies formed the Naphthalene Research Committee (NRC) to co-sponsor research that strives to improve naphthalene risk assessments. The NRC's objective is to reduce the use of default assumptions in assessing cancer risks potentially posed by exposure to naphthalene. To further its objective, the NRC funds research that is published in the peer-reviewed scientific literature. Results of several completed NRC sponsored studies could significantly change a quantitative risk assessment by replacing default values and identifying species-specific modes of action. Acute (single 6-hour) and 5 day exposure studies in rats demonstrated that high concentrations used in NTP two-year studies caused severe damage to the olfactory epithelium - a target tissue for subsequent tumor formation. Respiratory uptake studies of naphthalene in rats demonstrate that use of the Category 1 gas model is an inappropriate default assumption in assessing naphthalene risk. Results of a NRC 90-day exposure study establish a naphthalene NOEL in the rat, and demonstrate a remarkable degree of post-exposure recovery. Tissues collected following that 90-day exposure have been used in a p53 mutation study, genomics analysis and detailed metabolism mass balance and biomarker studies. NRC dose-response in vitro studies in various tissue types from rats, mice and humans demonstrate species and organ specific effects. A PBPK model incorporating naphthalene-specific data rather than regulatory default assumptions is nearing completion. Physiologic and metabolic differences between rodents and primates exposed to naphthalene introduce significant uncertainty into the applicability of studies conducted in rats and mice to determine human risk assessment. NRC is using the key events framework and the Hypothesis-Based Weight of Evidence paradigm to evaluate assumptions, alternative hypotheses, and guide development of data needed for a robust mode of action hypothesis.

PS 1958 PUTATIVE MECHANISMS OF ENVIRONMENTAL CHEMICAL-INDUCED STEATOSIS.

J. Kaiser. *Oak Ridge Institute for Science and Education, Oak Ridge, TN.*

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the U.S. occurring in nearly 30% of the population. NAFLD is characterized by several pathological changes with steatosis (or fatty liver) representing the initial step in its pathogenesis. Steatosis is of critical importance because the prevention of fatty liver can not only obviate the downstream pathologies of NAFLD (i.e. steatohepatitis, fibrosis, and cirrhosis) but also reduces the cardiometabolic risk associated with this form of liver disease. One of the major causes of NAFLD is thought to be obesity, but recent studies have shown a strong correlation between chemical exposures and fatty liver induction unrelated to being overweight. The U.S. EPA's Integrated Risk Information System (IRIS) is a human health assessment program that evaluates risk information on effects that may result from exposure to environmental contaminants. The goal of our work was to distinguish IRIS chemicals that induce steatosis and investigate putative mechanism(s) by which these chemicals may contribute to this hepatic pathological condition. Interestingly, a majority of steatosis-causing IRIS chemicals were chlorinated compounds and we hypothesize that they induce fatty liver by hepatic mitochondrial function impairment. Furthermore, the most toxic chemicals with respect to fatty liver induction (e.g., mirex, chlordane, chlordecone) had the greatest degree of chlorination. We also found that although it is likely important, mitochondrial dysfunction may not be the sole mediator of chemical-induced steatosis. Insulin resistance, impaired hepatic lipid secretion, and enhanced cytokine production were other potential mechanisms by which IRIS chemicals such as vinyl chloride, carbon tetrachloride, and alachlor, respectively, could contribute to hepatic steatosis. Taken together, the work described here is significant because it identifies multiple mechanisms by which specific IRIS chemicals may cause fatty liver and expands upon our knowledge of the possible role of environmental chemical exposure in the early progression of NAFLD.

PS 1959 POLYCHLORINATED BIPHENYLS ARE NOT "DIOXINS", AND OTHER INCONVENIENT TRUTHS RELEVANT TO THE USE OF TOXIC EQUIVALENCY FACTORS (TEFS) IN HUMAN HEALTH RISK ASSESSMENT.

J. B. Silkworth and E. A. Carlson. *General Electric, Niskayuna, NY.*

Various health effects have been documented following exposure to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The same cannot be said for similar exposures to polychlorinated biphenyls (PCBs), where any associated health effects have been attributed to non-PCB contaminants. So why are some PCB congeners referred to as "dioxin equivalents" in risk assessment? The answer lies in the perpetual use, for over seventy years, of ultra-sensitive animal models and PCB preparations of unknown purity to predict PCB toxicity to humans. This body of scientific literature is the basis of the current TEFs. There are a variety of reasons why the TEF scheme should not be employed to assess the risk of PCB mixtures to human health. First, PCBs are not "structural equivalents" of dioxins, cannot achieve rigid planar conformations, and interact within the aryl hydrocarbon receptor (AHR) ligand-binding domain (LBD) in a manner dissimilar to that of potent AHR agonists. This results in comparatively weaker binding affinities for PCBs and relegates most congeners as only partial agonists. In addition, differential recruitment of co-factors has been documented between PCBs and dioxins, possibly affecting AHR transactivation in a ligand-specific manner. Second, the human AHR differs significantly from those of sensitive animal species. An amino acid substitution in the human AHR LBD pushes its affinity for the most potent PCB (PCB 126) into the micromolar range. This genetic difference, along with even greater AHR protein degeneracy among species outside the LBD, appear to result in severely reduced efficacies for all PCB congeners in humans. Lastly, relative potencies for AHR ligands differ significantly between responsive species and humans in a congener-specific manner. This final fact has been proven for several PCB congeners by multiple investigators, using different cell types. Overall, congener- and species-specific variation in AHR activity clearly limits the applicability of TEFs for human health risk assessment.

PS 1960 APPLICATION OF LINEAR AND NON-LINEAR DOSE-RESPONSE MODELS FOR POLYCHLORINATED BIPHENYLS (PCBs) BASED ON A RECEPTOR-MEDIATED MODE OF ACTION (MOA) FOR TUMOR PROMOTION.

R. E. Keenan[1], P. O. Gwinn[1], J. M. Hamblen[1], J. Shoenfelt[2] and J. B. Silkworth[3]. [1]*Integral Consulting Inc., Portland, ME,* [2]*Integral Consulting Inc., Annapolis, MD and* [3]*General Electric Global Research, Niskayuna, NY.*

A nonlinear dose-response model for polychlorinated biphenyls (PCBs) was developed, based on a receptor-mediated mode-of-action (MOA) for PCB-promoted rat liver tumors. Tolerable Daily Intakes (TDIs) for several PCB mixtures and congeners, which had been tested in suitable lifetime rodent bioassays, were derived using the nonlinear model. The TDIs were then compared to risk-specific doses calculated using the linear default extrapolation approach that is currently used by EPA. The statistical dose-response analysis was conducted using EPA Benchmark Dose Software to model dose response in the observable region of the data in order to extrapolate to a point-of-departure (POD) at or near the fringe of the observable region. A set of conservative toxicological uncertainty factors (UFs) was used for extrapolating below the POD to the threshold dose anticipated by the MOA. This analysis led to the determination of TDIs that are substantially different than the corresponding risk-specific doses (10^{-4}-to-10^{-6} risk range) for each PCB mixture or congener. The nonlinear dose-response assessment indicates that much higher levels of PCBs can be tolerated without subjecting individuals to unreasonable excess cancer risks. These results have important regulatory policy implications for setting environmental tolerances and for determining acceptable cleanup levels of PCBs in soil, sediment, and water.

PS 1961 TOXIC EQUIVALENCY FACTORS ARE NOT UNIVERSAL WITHIN VERTEBRATE CLASSES: EVIDENCE, MECHANISTIC REASONS, AND REGULATORY IMPLICATIONS.

E. A. Carlson[1], C. H. Sutter[2], T. R. Sutter[2] and J. B. Silkworth[1]. [1]*General Electric, Niskayuna, NY and* [2]*University of Memphis, Memphis, TN.*

At least forty years ago, investigators realized that extreme differences in sensitivity to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related dioxin-like compounds (DLCs) exist among animal species. It was also recognized that these chemicals are invariably found in complex mixtures, leading to approaches for summarizing the toxic equivalency (TEQ) of such mixtures for risk assessment purposes. The World Health Organization (WHO) toxic equivalency factor (TEF) scheme, which compares individual DLC toxicity to that of TCDD, is now used for determining mixture TEQs. Although it was probably thought initially that species differences in sensitivity to TCDD and DLCs would be effectively normalized for congener-specific TEFs by generating relative potency (REP) estimates within experiments, the WHO panel soon realized that different vertebrate classes required different sets of TEFs. Sufficient evidence now exists that even within a vertebrate class, REPs are not universal. Species-specific REPs have been identified in experiments comparing responses of human and rodent cells, among avian species, and even among rodent strains. For example, our laboratory has demonstrated a human REP of 0.002 for 3,3',4,4',5-pentachlorobiphenyl (PCB 126), as determined by cytochrome P450 1A1 (CYP1A1) induction in fresh human hepatocytes and normal human epidermal keratinocytes (NHEKs), significantly lower than its assigned TEF of 0.1 (derived mainly from rodent studies). For fresh human and rat hepatocytes, this species difference extends to genes other than just CYP1A1 and is directly related to species differences in the aryl hydrocarbon receptor pathway. Furthermore, using NHEKs, we have demonstrated that such species difference extends to other congeners. Identified species- and congener-specific REPs are robust, repeatable, and significant. Such differences call into question the indiscriminant use of TEFs to predict risk in a species from which they were not derived.

PS 1962 LOW DOSE RESPONSE RELATIONSHIPS FOR NON-CANCER ENDPOINTS: OZONE, MERCURY, AND XENOESTROGENS.

L. Zeise[2], G. Ginsberg[1] and M. S. Sandy[2]. [1]*Connecticut Department of Public Health, Hartford, CT and* [2]*Cal EPA Office of Environmental Health Hazard Assessment, Oakland, CA.*

The 2009 National Academy of Sciences report "Science and Decisions: Advancing Risk Assessment" describes different approaches to modeling dose response relationships – that depend on background exposures (endogenous and exogenous) of chemicals that contribute to the disease process, ongoing disease processes, commonness of the disease, and sensitive and vulnerable populations. These factors are explored with three chemical substances: Ozone, mercury and xenoestrogens. While potential mechanisms of action may suggest low dose non-linearities at the individual level (due for example to host defense), this cannot be generalized, because of variability in exposures and ongoing health and disease processes that can translate to linear dose response at the population level. Acute, clinical ozone exposure studies in humans provide evidence of a linear dose response in FEV1 at low doses. These and other studies suggest that commonly experienced concentrations are still above the threshold for toxicity, at even the individual level for healthy adults for some endpoints. For methylmercury neurodevelopmental effects, modeling and data suggest low dose linearity at the population level. We explore the case of mercury further by considering the limited epidemiology studies for Hg° from dental amalgams, potential impacts of methylmercury on low dose observations of Hg° effects, and other factors. Integration of animal data into low dose modeling of Hg° effects is explored. For xenoestrogens, background exposures and disease processes suggest low dose linearity for a variety of effects. These case studies suggest linear dose response at the population level for environmental exposures, similar to what has been found for particulate matter and lead.

PS 1963 ANALYZING THE SAFETY OF GULF SEAFOOD AFTER THE OIL SPILL BASED ON POPULATION SPECIFIC EXPOSURE SCENARIOS AND INCORPORATION OF DISPERSANT AND HEAVY METAL TOXICITY PARAMETERS.

J. M. Gohlke. *Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL.*

Gulf fishermen are facing a number of challenges since the British Petroleum Deepwater Horizon explosion, oil spill and cleanup. One challenge is re-instating consumer confidence in their product. Since the re-opening of closed Gulf waters to fisheries, numerous groups, including members of Congress, non-governmental organizations, scientists, local fisherman, processors, and chefs, have raised concerns over the adequacy of the National Oceanic and Atmospheric Administration (NOAA) and Food and Drug Administration (FDA) protocol for ensuring the safety of seafood caught in the Gulf. Primary concerns raised include reliance on an initial smell test and chemical testing only for a select number of polycyclic aromatic hydrocarbon (PAH) components of oil, as well as questions regarding the sampling scheme and risk assessment methodology employed. There is considerable uncertainty regarding the potential for toxicity related to the use of Corexit 9500A and Corexit 9527, the primary dispersants employed to break up the oil in the Gulf. For example, the potential for bioaccumulation of components of the dispersant in finfish, particularly those that are long-lived and at the top of the food chain is uncertain. Large amounts of heavy metals have also been released into the Gulf. Recent research suggests oil spills alter natural filtration processes of arsenic, raising the concern that increased levels of arsenic exposure via seafood consumption may be an issue. This project details assumptions in the NOAA/FDA methodology and develops region specific exposure assessments reflecting local consumption patterns, particularly in potentially vulnerable populations of subsistence fisherman, pregnant women and young children. In addition, the project focuses on risk characterization methodologies that estimate composite risk of cancer and non-cancer endpoints based on toxicity of dispersant components, heavy metal exposure, as well as relevant PAHs, resulting in novel estimates of levels of concern (LOCs).

PS 1964 CORN OIL AS A CAUSATIVE FACTOR FOR PROLIFERATIVE LESIONS OF THE FORESTOMACH IN B6C3F1 MICE EXPOSED BY GAVAGE.

L. M. Plunkett[1], T. Starr[2], J. A. MacGregor[3] and A. M. Jonyas[4]. [1]*Integrative Biostrategies LLC, Houston, TX,* [2]*TBS Associates, Raleigh, NC,* [3]*Toxicology Consulting Services, Arnold, MD and* [4]*Amvac Chemical Corporation, Newport Beach, CA.*

A National Toxicology Program (NTP) cancer bioassay has established that the dose of corn oil that is routinely administered daily in gavage studies (5 ml/kg for F344 rats) is by itself associated with altered tumor incidence rates. Thus, corn oil is a potential confounding factor in carcinogenicity testing/ data interpretation. While mice receive twice the rat dose (10 ml/kg) in NTP corn oil gavage studies, a similar study of corn oil effects in B6C3F1 mice has not been conducted. Our previous work has implicated daily administration of corn oil by gavage as a causative factor for proliferative lesions of the forestomach in B6C3F1 mice. In the present analysis, the role of corn oil was examined by comparing tumor incidence rates in mice exposed to corn oil or water via gavage. A rigorous statistical assessment of heterogeneity was conducted using mouse forestomach hyperplasia and tumor incidence rates as reported for control groups of mice in the NTP bioassay series. Results were compared with those from our earlier analyses that assessed heterogeneity among control group incidence rates for studies using dietary and inhalation exposure routes. The combined analyses reveal significantly lower incidence rates of forestomach lesions in control groups of mice exposed via water gavage, the diet, or by inhalation, as compared to the rates of these lesions in corn oil gavage studies. The combined analyses show that daily gavage with corn oil can be a causative factor for proliferative forestomach lesions in B6C3F1 mice, and that corn oil by itself is a significant contributing factor in mouse forestomach lesion development.

PS 1965 ASSESSING RISKS FOR SEC- AND TERT-BUTYLBENZENE: A SURROGATE METHOD.

N. Y. Wang[1] and C. J. Moudgal[2]. [1]*National Center for Environmental Assessment, U.S. EPA, Cincinnati, OH and* [2]*Environmental Risk & Toxicology, ICF International, Dublin, CA.*

As part of the U.S. Environmental Protection Agency's (U.S. EPA) mission to protect the environment, chemicals-of-concern (CoCs) at Superfund or other hazardous waste sites are cleaned up based on their potential toxicity to humans and the surrounding ecosystem. However, often CoCs have limited or no toxicity data to assess their impacts on human health or the ecosystem. In these instances the application of alternative approaches, such as Structure-Activity Relationship (SAR) can be considered in conjunction with supporting data such as toxicokinetic and chemical class-specific toxicity data to identify suitable surrogates for CoCs. sec- and tert-Butylbenzenes are two CoCs from the alkylbenzenes class of chemicals that have very little relevant toxicity data for risk assessment purposes. We propose a SAR-based surrogate application for sec- and tert-butylbenzenes by using a combination of toxicokinetic and class-specific toxicity data. Three potential structural and metabolic surrogates for sec- and tert-butylbenzene were identified: ethylbenzene, n-butylbenzene, and isopropylbenzene. While these potential surrogates exhibited some similarity in toxicokinetic characteristics and toxicity manifestation, the branched alkylbenzenes, which include isopropylbenzene and sec- and tert-

butylbenzenes, exhibited most similar patterns in absorption, distribution and excretion as well as metabolic activity. Additionally, the acute toxicities of branched alkylbenzenes were reported to be higher in various tissues compared to their linear counterparts such as ethylbenzene and n-butylbenzene. A similarity search using U.S. EPA's DSSTox database and National Library of Medicine (NLM) ChemIDPlus also support isopropylbenzene as an appropriate surrogate for sec- and tert-butylbenzene. Based on this weight-of-evidence analysis, toxicity data on isopropylbenzene may serve as a surrogate for both sec- and tert-butylbenzenes.

PS 1966 EVALUATING *IN SILICO* AND *IN VITRO* TECHNOLOGIES FOR PREDICTING HEPATOTOXICITY.

W. J. Bailey[3], A. Bahl[1], E. Keough[2], K. R. Leander[2], C. Gretzula[4], I. Pak[1], M. C. Kuhls[3], C. Kreatsoulas[5], R. P. Sheridan[5], A. G. Aslamkhan[3], L. Kiss[4], A. Bagchi[1] and B. J. Howell[2]. *[1]Informatics IT, Merck & Co., Inc., West Point, PA, [2]RNA Therapeutics, Merck & Co., Inc., West Point, PA, [3]Analytical & Systems Toxicology, Merck & Co., Inc., West Point, PA, [4]in vitro Pharmacology, Merck & Co., Inc., West Point, PA and [5]Chemistry Modeling & Informatics, Merck & Co., Inc., West Point, PA.*

In vitro predictive toxicology strategies deployed in the hit-to-lead space can serve to enrich the candidate pool for less toxic compounds, thus driving down the cost and uncertainty of drug development. As hepatotoxicity is a major source of attrition during preclinical and clinical development, we focused on developing and evaluating different technologies (in silico and in vitro) for building classifiers that predict dose-dependent liver injury. The in silico approach utilizes predicted physical properties (PPP) derived from chemical structures alone (e.g. lipophilicity), thereby providing an assay-free means to guide compound choices very early in the drug discovery pipeline. Among the two in vitro technologies that we studied, high-throughput screens (HTS) interrogate select toxicological biomarkers in a liver cell line upon chemical perturbation using simple assays amenable to automation (e.g. cell viability), allowing deployment in pipeline phases expecting a routinely high flux of compounds to be tested. The second in vitro technology examined, high-content screening (HCS), is an image-based methodology for profiling cellular effects across a spectrum of chosen hepatotoxicity endpoints simultaneously in individual cultured liver cells upon compound treatment, providing richer information than HTS assays that is useful for mechanistic dissection, but which comes with an associated increase in assay complexity that limits throughput. In this study, we present the cost, complexity, throughput, and performance profiles of PPP-, HTS-, and HCS-based predictive toxicology approaches benchmarked on an internal Merck compound set where in vivo liver histopathology endpoints are known.

PS 1967 CANCER HAZARD IDENTIFICATION UTILIZING STRUCTURE-ACTIVITY CONSIDERATIONS FOR 1, 3-DICHLOROPROPANOL AND 3-MONOCHLOROPROPANE-1, 2-DIOL.

D. W. Morry, R. S. Tomar, F. C. Tsai, M. S. Sandy and L. Zeise. *Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA.*

1,3-Dichloropropanol (1,3-DCP), a high production volume chemical, and 3-monochloropropane-1,2-diol (3-MCPD), a rodenticide, are chlorinated three-carbon alcohols that are also found in certain processed foods. These two compounds were recently added to the Proposition 65 list of chemicals known to the State of California to cause cancer. Here we focus on the structure-activity information that contributed to the evidence used to identify these compounds as carcinogens. We compare 1,3-DCP and 3-MCPD with seven related three-carbon compounds and two phosphate triesters that are metabolized to three-carbon halogenated alcohols. The three-carbon compounds are: 2,3-dibromo-1-propanol, epichlorohydrin, glycidol, 1,3-dichloroacetone, 1,2,3-trichloropropane, 1,3-dichloropropene (Telone II), and 1,2-dibromo-3-chloropropane. The phosphate triesters are: tris(1,3-dichloro-2-propyl)phosphate (TDCPP) and tris(2,3-dibromopropyl)phosphate (TDPP). All of these compounds have positive evidence of genotoxicity, and all but two (1,3-dichloroacetone, TDCPP) have been identified as carcinogens by IARC or Proposition 65. The rodent carcinogenicity data on these chemicals indicate that they are capable of inducing tumors at multiple sites. Many of these chemicals induce tumors at one or more of the same sites as 1,3-DCP and 3-MCPD. Several mechanisms have been proposed to explain how these eleven compounds induce tumors, including genotoxicity, possibly via formation of glutathione metabolites capable of forming DNA adducts. We conclude that structure-activity considerations can provide valuable information for the assessment of cancer hazard.

PS 1968 ARSENIC INDUCES METABOLIC REGULATORS AND DIFFERENTIATION IN ADIPOSE TISSUE.

L. R. Klei, Y. Garciafigueroa, R. T. Cattley and A. Barchowsky. *Environmental and Occupational Health, Universtity of Pittsburgh, Pittsburgh, PA.*

Consumption of low to moderate levels of arsenic promotes a number of diseases that stem from altered metabolism, such as cardiovascular disease and diabetes. The patterns of diseases suggest enhanced metabolic syndrome, but underlying mechanisms are less clear. To investigate potential mechanisms, we explored the hypothesis that arsenic exposure promotes pathogenic phenotypic or functional change in mouse adipose tissues. C57Bl/6 mice were assessed for circulating lipid levels and adipose tissue expression of regulators of lipid and energy metabolism. Decreased serum triglyceride was the only observable circulating lipid change. Analysis of RNA and protein extracts isolated from interscapular brown adipose tissue (BAT) demonstrated up regulation of transcriptional programs for thermogenesis with increased expression of PGC-1α, PnPla2, UCP1, PPARγ, and PPARδ. Interestingly, transcript levels for a number of inflammatory mediators (CRP, Ccl3, IL6, and vaspin) decreased in BAT. Epididymal white adipose tissue (WAT) demonstrated similar patterns of change, especially with increases in PGC-1α and UCP1 at both the transcript and protein levels. These regulators of BAT thermogenesis are normally very lowly expressed in WAT and the expression indicates WAT acquiring BAT character. This phenotypic change would be supported by the increased markers of adipose stem cell differentiation, such as PPARγ. Increased PECAM1, IGF1, and VEGF indicated enhanced WAT vascularization. Interestingly, many adipose genes, such as visfatin, leptin, lipoprotein lipase, and adipophilin were unchanged. However, PEPCK, the rate limiting step in gluconeogenesis, levels were increased. Together, the pattern of arsenic-related change in adipose tissues explain the decrease in serum triglycerides, but disrupted metabolism with enhanced potential for released glucose. Further investigation is warranted to determine the full impact of these metabolic changes to etiology of arsenic related metabolic diseases. Supported by NIEHS grant R01ES013781.

PS 1969 TRANSCRIPTIONAL REPRESSOR INVOLVED IN GLUCOSE METABOLISM PLAYS A KEY ROLE IN PROTECTION AGAINST ARSENITE TOXICITY.

T. Takahashi, N. Miyanaga, T. Yano and A. Naganuma. *Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi, Japan.*

Arsenic is known to be an environmental toxic metalloid that causes various cancers and skin disorders, but its mechanism of toxicity are not yet clearly understood. To elucidate more detail mechanism of arsenic toxicity, we have previously searched for factors that determine sensitivity of cells to arsenite using budding yeast as a model eukaryote and found that disruption of Reg1, a regulatory subunit of protein phosphatase 1 (PP1), increased sensitivity of yeast cells to arsenite. In this study, we found that the deletion of Reg1 increased sensitivity of yeast cells to arsenite through activation of Snf1 kinase. Therefore, we investigated the relationship between downstream factors of Snf1 and arsenic toxicity and found that deletion of Mig1, a transcriptional repressor involved in glucose metabolism, conferred hypersensitivity to arsenite. Mig1 is known to be down-regulated by Snf1 via phosphorylation. Simultaneous deletion of Reg1 and Mig1 showed no additive or synergistic effects on sensitization to arsenite, suggesting that sensitization of yeast cells to arsenite by deletion of Reg1 might be involved in depression of Mig1 by Snf1. On the other hand, arsenite enhanced phosphorylation of Mig1 and decreased the transcriptional repressor activity of Mig1. These results suggest that inactivation of Mig1 via phosphorilation might be involved in development of arsenic toxicity.

PS 1970 CHRONIC EXPOSURE TO MODERATE DOSES OF ARSENIC INCREASES ATHEROSCLEROSIS FORMATION OF UNSTABLE PLAQUES.

M. Lemaire[1], C. A. Lemarié[2], M. Flores-Molina[1], E. L. Schiffrin[2], S. Lehoux[2] and K. K. Mann[1]. *[1]Oncology, McGill University, Montreal, QC, Canada and [2]Medicine, McGill University, Montreal, QC, Canada.*

Arsenic is a widespread environmental contaminant to which millions of people are exposed worldwide. The current maximum acceptable level of arsenic in municipal water is set at 10 ppb, but well water in many North American counties averages 10-fold higher than this limit. Arsenic exposure has been linked to atherosclerosis, however molecular mechanisms involved or minimal required doses are unknown. Therefore, we assessed the effects of a chronic exposure to moderate concentrations of arsenic on plaque formation and plaque composition in the ApoE-/- murine atherosclerosis model. We exposed ApoE-/- mice to two doses of arsenic (200 ppb or

1000 ppb) for either 8 or 13 weeks in presence or absence of high fat diet. Cholesterol levels and body weight were monitored and were not significantly altered with arsenic exposure. However, we observed a significant increase in plaque size in mice exposed to the lower dose of arsenic, compared to either control, unexposed mice or mice exposed to the higher dose of arsenic. Moreover, arsenic exposure altered plaque composition, by increasing lipid deposition and decreasing smooth muscle cell and collagen content. These modifications characterize less stable lesions, which are prone to rupture and thus, more dangerous. Our observations strongly suggest that chronic exposure to moderate doses of arsenic predispose to a greater cardiovascular risk than previously anticipated.

PS 1971 LOW DOSE ARSENIC EXPOSURE INCREASES MONOCYTE AND MACROPHAGE ADHESION AND PRO-ATHEROGENIC PHENOTYPE.

M. Flores-Molina, M. Lemaire, C. A. Lemarié, S. Lehoux and K. K. Mann. *McGill University, Montreal, QC, Canada.*

Environmental arsenic exposure is linked epidemiologically to increased atherosclerosis. However, the mechanisms by which arsenic enhances atherosclerosis and the minimal dose at which arsenic exposes subjects to risk are unknown. Monocytes and macrophages are key players in the early stages of atherosclerotic lesion formation. They bind to the activated endothelium of vessels and migrate in the sub-endothelium where they become lipid laden and form foam cells. Therefore, we assessed the effects of a low-to-moderate exposure to arsenic on monocyte adhesion to the vascular wall. We first tested whether arsenic modulates the binding of monocytes to the adhesion molecule VCAM-1, found on activated endothelial cells. Our results showed that 1) arsenic exposure increased the adhesion of both U937 monocytic cells and primary human monocytes or macrophages to VCAM-1; 2) arsenic exposure increased protein and surface expression of the β1 integrin, which binds to VCAM-1, on monocytes and macrophages; and 3) the low-to-moderate doses of arsenic (10-200 ppb) had a more potent effect than the highest dose (1000 ppb). We extended these results using an ex vivo organ culture system of murine carotids arteries. We found that arsenic exposure increased the adhesion of murine bone marrow cells to the endothelium of the carotid arteries in a dose-dependent manner, when cells and vessels were treated separately. Furthermore, when vessels and bone marrow-derived cells were both exposed to 10 ppb arsenic, there was a significant increase in cells attached to the endothelium. Together, these data suggest that arsenic may promote atherosclerosis by increasing cell adhesion to the vascular wall. Moreover, these effects are observed at concentrations of arsenic that are approaching the maximum contaminant level (10 ppb) recommended by the World Health Organization for municipal water. Our data clearly show that arsenic may be a greater health risk than previously anticipated.

PS 1972 BIOMARKERS FOR THE EARLY DETECTION OF ATHEROSCLEROSIS IN A CHILDREN POPULATION ENVIRONMENTALLY EXPOSED TO INORGANIC ARSENIC.

C. Osorio-Yáñez[1], J. C. Ayllon-Vergara[2], L. Arreola-Mendoza[1, 3], E. Hernández-Castellanos[1], M. A. Sanchez-Guerra[1], E. M. Melgar-Paniagua[1], A. De Vizcaya-Ruiz[1], G. Aguilar-Madrid[4] and L. M. Del Razo[1]. [1]*Toxicology, Cinvestav-IPN, Mexico D.F., Mexico,* [2]*Hospital Español, Mexico, Mexico,* [3]*Biosciences and Engineering, CIIEMAD-IPN, Mexico, Mexico and* [4]*Health and Work, IMSS, Mexico, Mexico.*

Atherosclerosis is a multistage disease that can initiate at childhood and progresses for decades as a silent process until adult age, when the clinical manifestations occur. There is a growing interest to prevent cardiovascular disease early in the course of the disease even at a pediatric stage. Up-regulation of endothelial adhesion molecules, including soluble intercellular cell adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1), play a pivotal role in the earliest phases of atherosclerosis by mediating the binding and subsequent recruitment of monocytes into arterial intima, triggering lipid deposits and streaks. Increase in carotid intima-media thickness (cIMT) is an established marker for early preclinical atherosclerosis. In this cross-sectional study we examined the serum concentration of endothelial dysfunction biomarkers (sICAM-1, sVCAM-1) and cIMT in 198 children (3-8 years old), residents of Zimapan, Mexico who are chronically exposed to inorganic arsenic (iAs) through drinking water. Our results show a significant positive correlation between sICAM-1 and sVCAM-1 (r=0.5022; p=0.0001), and notably, a significant relationship was observed between cIMT and sICAM-1 (r=0.186; p=0.035). Similarly, sVCAM-1 and sICAM-1 showed a positive significant correlation with atherogenic lipids as VLDL and triglycerides. Endothelial adhesion molecules and cIMT may be related to early atherosclerosis in children chronically exposed to iAs. (Funded by Conacyt Mexico).

PS 1973 MITOGEN ACTIVATED PROTEIN KINASE PATHWAY IS INVOLVED IN THE UP-REGULATION OF KERATIN 6 IN ARSENITE AND CADMIUM TRANSFORMED UROTSA CELLS.

S. Somji, L. Cao, S. H. Garrett, M. Sens and D. A. Sens. *Pathology, University of North Dakota, Grand Forks, ND.*

Arsenite (As^{3+}) and cadmium (Cd^{2+}) are known carcinogens that have been implicated in the development of bladder cancer. Previous work from this laboratory has shown that normal urothelial cells (UROtsa) do not express keratin 6, however malignant transformation of these cells by the heavy metals As^{3+} and Cd^{2+} results in an over expression of keratin 6. The tumor heterotransplants generated from these cells also expressed keratin 6 which was localized to areas of the tumor that had squamous differentiation. Additional studies performed in our laboratory suggested that epidermal growth factor (EGF) and insulin may be involved in the up-regulation of keratin 6 in the normal UROtsa parental cell lines. The goal of this study was to determine if acute and chronic exposure to the heavy metals As^{3+} and Cd^{2+} could induce the expression of keratin 6 in the parental UROtsa cell line. Furthermore, we were interested in determining the mechanism involved in the up-regulation of keratin 6 in the parental as well as the transformed cell lines. For this purpose, the UROtsa cells were exposed to various concentrations of As^{3+} and Cd^{2+} for various time periods and the expression of keratin 6 was determined. The results obtained from our studies suggest that acute and chronic exposure to As^{3+} and Cd^{2+} does not induce the expression of keratin 6 in the UROtsa cells. In order to study the signal transduction mechanism involved in the regulation of expression of keratin 6, the parental and the transformed cells were exposed to EGF or insulin for various time periods and western blots were performed on the cell extracts with antibodies specific for factors involved in the mitogen activated protein kinase pathway. The data obtained suggests that the ERK1/2 pathway is involved in the up-regulation of keratin 6 in the parental as well as transformed UROtsa cells. Inhibition of MEK1 and MEK2 by the inhibitor U0126 resulted in a decreased expression of keratin 6 further implicating this pathway in the up-regulation of keratin 6.

PS 1974 SEAFOOD INTAKE AND URINE CONCENTRATIONS OF TOTAL ARSENIC, DIMETHYLARSINATE, AND ARSENOBETAINE IN THE U.S. POPULATION.

A. Navas-Acien[1, 2], K. A. Francesconi[3], E. K. Silbergeld[1] and E. Guallar[2]. [1]*Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD,* [2]*Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD and* [3]*Institute of Chemistry, Karl-Franzens University Graz, Graz, Austria.*

Background: Seafood is the main source of organic arsenic exposure (arsenobetaine, arsenosugars and arsenolipids) in the population. Arsenosugars and arsenolipids are metabolized to several species including dimethylarsinate (DMA). Objective: Evaluate the association of seafood intake with spot urine arsenic concentrations in the 2003-2006 National Health Nutrition and Examination Survey (NHANES). Methods: We studied 4276 participants ≥6 y. Total arsenic was measured using inductively coupled plasma dynamic reaction cell mass spectrometry (ICPMS). Urine DMA and arsenobetaine were measured by high-performance liquid chromatography coupled with ICPMS.
Results: Participants reporting seafood in the past 24-h had higher urine concentrations of total arsenic (median 24.5 vs. 7.3 μg/L), DMA (6.0 vs. 3.5 μg/L), arsenobetaine (10.2 vs. 0.9 μg/L) and total arsenic minus arsenobetaine (11.0 vs. 5.5 μg/L). Participants reporting seafood ≥2/wk vs. never during the past year had 2.3 (95% confidence interval 1.9, 2.7), 1.4 (1.2, 1.6), 6.0 (4.6, 7.8) and 1.7 (1.4, 2.0) times higher (p-trend <0.001) concentrations of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine, respectively. In participants without detectable arsenobetaine and in analyses adjusted for arsenobetaine, seafood consumption in the past year was not associated with total arsenic or DMA concentrations in urine. Conclusion: Seafood intake was a major determinant of increased urine concentrations of total arsenic, DMA, arsenobetaine and total arsenic minus arsenobetaine in the US population. Human studies that use total arsenic, DMA, the sum of inorganic arsenic, methylarsonate and DMA, and total arsenic minus arsenobetaine as markers of inorganic arsenic exposure and/or metabolism need to address seafood intake.

PS 1975 DIFFERENTIAL MODULATION OF CYP1A1 BY ARSENITE *IN VIVO* AND *IN VITRO* IN C57BL/6 MICE.

A. Anwar-Mohamed and A. O. El-Kadi. *Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.*

Arsenite (As(III)), have been implicated in altering the carcinogenicity of aryl hydrocarbon receptor (AhR) ligands, typified by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), via modulating the induction of the cytochrome P450 1a1 (Cyp1a1) enzyme, but the mechanism(s) remain unresolved. In this study, the effect of As(III) on Cyp1a1 expression and activity was investigated in C57BL/6 mice livers and isolated hepatocytes. For this purpose, C57BL/6 mice were injected intraperitoneally with As(III) (12.5 mg/kg) in the absence and presence of TCDD (15 μg/kg). After 6 or 24 h, livers were excised and the levels of Cyp1a1 mRNA, protein, and catalytic activity levels were determined using real time-PCR, Western blot, and 7-ethoxyresorufin-O-detethylase (EROD) analyses, respectively. At in vitro level, isolated hepatocytes from C57BL/6 mice were treated with increasing concentrations of As(III) (1, 5, and 10 μM) in the absence and presence of TCDD (1 nM) for different time-points (0 - 24 h). Our results showed that at in vivo level, As(III) decreased the TCDD-mediated induction of Cyp1a1 mRNA at 6 h while potentiating the TCDD-mediated induction of Cyp1a1 mRNA, protein, and catalytic activity levels at 24 h. In isolated mouse hepatocytes, As(III) decreased the TCDD-mediated induction of Cyp1a1 mRNA in a time-dependent manner. Moreover, As(III) decreased the TCDD-mediated induction of Cyp1a1 protein and catalytic activity levels at 24 h. Investigating the effect of co-exposure to As(III) and TCDD at transcriptional levels revealed that As(III) significantly inhibited TCDD-mediated induction of AhR-dependent luciferase reporter gene expression. In conclusion, caution should be taken when extrapolating in vitro data to in vivo situation. Furthermore, our results denote more complex regulation of Cyp1a1 at the in vivo level that warrants further investigation. Supported by Natural Sciences and Engineering Research Council of Canada Discovery Grant RGPIN 250139-07.

PS 1976 ROLE OF CONTINUOUS LOW-LEVEL MONOMETHYLARSONOUS ACID EXPOSURE IN THE INHIBITION OF PARP AND CONTRIBUTION TO INCREASED GENOTOXICITY IN THE MALIGNANT TRANSFORMATION OF UROTSA CELLS.

S. M. Wnek, M. M. Medeiros, J. M. Camarillo, T. J. Jensen, X. Zheng, B. W. Futscher and A. Gandolfi. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Exposure of human bladder cells (UROtsa) to 50 nM of the arsenic metabolite, monomethylarsonous acid, (MMAIII) for 12 wk results in malignant transformation and a time-dependent increase in DNA damage. Acute studies have suggested an indirect role of arsenicals in eliciting genotoxicity; however, it is unknown whether continuous low-level MMAIII exposure can increase genotoxic potential by inhibiting DNA repair processes. Poly(ADP-ribose) polymerase-1 (PARP-1), a zinc finger protein, is rapidly activated upon DNA damage. However, in MMAIII-exposed UROtsa cells, PARP activity does not increase despite the increase in DNA damage through 12 wk of exposure; when UROtsa cells are removed from MMAIII exposure (2 wk), PARP activity increases and DNA damage levels decrease. Interestingly, PARP-1 gene and protein levels are elevated in the presence of MMAIII, possibly as a means to compensate for the decrease in global protein poly(ADP-ribosyl)ation. PARP plays a critical role in the cellular response to DNA damage and maintaining genomic stability; overall, these data suggest a potential role of MMAIII in the inhibition of PARP. The zinc finger domains of PARP contain vicinal sulfhydryl groups as a result of closely spaced cysteine amino acids, which may act as a potential site for MMAIII to bind PARP, displace zinc ion, and render PARP inactive. In the presence of continuous MMAIII exposure, zinc supplementation (4 wk) was able to increase PARP activity levels and reduce the genotoxicity associated with MMAIII, suggesting the possible interference of MMAIII with PARP zinc-finger motifs. Overall, these results present a potential mechanism in which MMAIII may inhibit PARP through the displacement of zinc; thus inactivating PARP and increasing the susceptibility of UROtsa cells to genotoxic insult/malignant transformation as well as explain the increased genotoxicity associated with methylated arsenicals when compared to inorganic arsenic.

PS 1977 P53 RESPONSE TO MONOMETHYLARSONOUS ACID EXPOSURE IN HUMAN BLADDER EPITHELIAL CELLS.

M. K. Medeiros and A. Gandolfi. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Epidemiological data has established a connection between the development of bladder cancer and chronic arsenic exposure. Acute effects of arsenical exposure have been well characterized with immortalized human urothelial cells derived from the lining of the ureter. In addition, chronic exposure of these immortalized cells to arsenicals has led to their malignant transformation. In this study, primary human bladder epithelial (HBE) cells were acquired commercially and maintained in a proprietary culture media designed to delay differentiation and senescence. A similar sensitivity to acute exposure of sodium arsenite and monomethylarsenous acid [MMA(III)], a highly toxic metabolite of arsenite, was observed when compared to immortalized urothelial cells (UROtsa). Chronic exposure of HBE to MMA(III) over a period of one year did not lead to a malignant transformation. HBE do express functional p53. With acute (3 weeks) exposure to 50 nM MMA(III), p53 and p21 expression is induced in response to DNA damage measured by the comet assay. To emulate the effects of the oncogenic alterations observed in UROtsa, HBE cells were transfected with a HPV E6 oncogene. The HBE(E6) cells demonstrated attenuation of p53 responses to etoposide and these modified cells have not shown anchorage-independent growth. This model will be subjected to chronic arsenic exposure and will hopefully serve to illustrate the important role of a dysfunctional p53 in arsenic carcinogenesis.

PS 1978 *IN VIVO* CONSEQUENCES OF ARSENIC METHYLATION IN A *DROSOPHILA* MODEL.

I. Cartwright[1], J. Muñiz Ortiz[2] and J. Shang[1]. [1]*Molecular Genetics, University of Cincinnati College of Medicine, Cincinnati, OH and* [2]*NHEERL, EPA, Research Triangle Park, NC.*

Metabolism of inorganic arsenic (As) into methylated derivatives by vertebrates was originally presumed to be a detoxification that facilitates export of As from the cell. However, recent analyses revealed that methylation may be a critical step underlying the toxicity of low level, chronic inorganic As exposure, e.g., in the development of various organ cancers. *Drosophila*, unlike vertebrates, does not possess As(III) methyltransferase (AS3MT), the enzyme mainly responsible for vertebrate As methylation. We introduced the human AS3MT gene into flies under conditions where we can obtain inducible As methylation *in vivo*. When transgenic flies induced to express AS3MT were exposed to inorganic arsenite-containing food at low concentrations (levels that do not produce overt toxicity), chromosomal instability was significantly enhanced, as determined by a loss of heterozygosity assay, compared to both wild-type and uninduced transgenic flies similarly exposed. Interestingly, when such transgenic flies were fed arsenite at significantly higher concentrations, enough to produce overt, quantifiable toxic effects on viability, we found that induction of AS3MT led to a significantly increased lifespan as compared to uninduced or non-transgenic wild type flies similarly exposed. Together, these experiments shed interesting light on the benefits, or otherwise, to an organism of As methylation. During acute exposure the capacity to methylate As is beneficial, presumably because it facilitates export, thereby lowering the cellular arsenic burden and promoting viability, at least in the short term. By comparison, chronic low-level As exposure does not precipitate immediate life-threatening effects, but methylation of As clearly promotes chromosome instability, which may, over time, predispose to cancer. Teasing out the molecular pathways intersected by As in these situations should be highly facilitated by pursuing the type of genetic analysis for which *Drosophila* is so renowned.

PS 1979 ASSOCIATION OF ARSENIC EXPOSURE AND GENETIC POLYMORPHISMS WITH ARSENIC METABOLISM IN A VIETNAMESE POPULATION.

T. Agusa[1, 2], T. Kunito[3], J. Fujihara[1], H. Takeshita[1], T. B. Minh[4], P. K. Trang[4], P. H. Viet[4], S. Tanabe[2] and H. Iwata[2]. [1]*Shimane University Faculty of Medicine, Izumo, Japan,* [2]*Ehime University, Matsuyama, Japan,* [3]*Shinshu University, Matsumoto, Japan and* [4]*Vietnam National University, Hanoi, Viet Nam.*

The present study investigated the arsenic (As) exposure and association of genetic polymorphisms in glutathione S-transferase (GST) family (GSTO1, O2, M1, P1, and T1) and arsenic (+3 oxidation state) methyltransferase (AS3MT) with As metabolism in 190 subjects from the As-contaminated groundwater areas in the Red River Delta, Vietnam. Concentrations of arsenite (As[III]), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in human urine were positively correlated with total As levels in the groundwater, suggesting that people living in these areas may be exposed to As through the groundwater. The concentration ratio of urinary DMA/MMA, which is an indicator of second methylation capacity, increased with the urinary As level. The wild type of GSTM1, hetero types of GSTO1 Glu155del and GSTP1 Ile105Val, and variant homo type of GSTO2 Asn142Asp had high MMA/inorganic As (IA; As[III] + arsenate (As[V])) ratios. Higher DMA/MMA was found in the wild types of AS3MT Met287Thr and GSTM1. The concentration ratio of As[III]/As[V] in the urine of the hetero type of GSTP1 Ile105Val and the wild type of GSTM1 was significantly higher than that of other genotypes in each gene. Interestingly, a positive correlation between

MMA/IA and urinary As concentration of the hetero type of GSTP1 Ile105Val was more significant than that of the wild type. Increased DMA/MMA with an increase in urinary As concentration was observed for the GSTM1 wild type but not for the null. Although the relationship between As[III]/As[V] and As concentration in the urine showed a significant positive correlation in the wild type of GSTP1 Ile105Val, it was not significant in the hetero type. For GSTO1 Ala140Asp, the heterozygote was associated with larger coefficients between As[III]/As[V] and urinary As level than the homozygote. These results suggest that the metabolism of As are affected by both As exposure and genetic polymorphisms.

PS 1980 AS3MT/M287T POLYMORPHISM IS A RISK FACTOR FOR DIABETES ASSOCIATED WITH CHRONIC EXPOSURE TO INORGANIC ARSENIC.

D. Loomis[1], L. M. Del Razo[2], G. G. Garcia-Vargas[3], Z. Drobná[4] and M. Styblo[4]. *[1]Medical Center, University of Nebraska, Omaha, NE, [2]Department of Toxicology, CINVESTAV-IPN, Mexico, Mexico, [3]Faculty of Medicine, Juarez University of Durango State, Gomez-Palacio, Mexico and [4]Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Our recent work in Zimapan and Lagunera regions in Mexico has linked chronic exposure to inorganic arsenic (iAs) in drinking water to an increased risk of diabetes. We have also shown that this risk is associated with increased urinary concentrations of dimethylarsinite (DMAsIII), a toxic methylated metabolite of iAs. Arsenic (+3 oxidation state) methyltransferase (AS3MT) is the key enzyme in the pathway for iAs methylation. AS3MT expression and polymorphism is, in part, responsible for inter-individual differences in iAs metabolism. We have, therefore, examined associations between AS3MT polymorphism, iAs metabolism and risk of diabetes among Zimapan and Lagunera residents. Our results show that AS3MT/287T genotype was associated with a higher average fasting blood glucose (FBG = 114 ±59 mg/dl) as compared to a more common AS3MT/287M genotype (89.85 ±35 mg/dl; p = 0.006) and with an increase in 2-hour blood glucose (2HBG) recorded during oral glucose tolerance test (135 ±98 vs. 103 ±46 mg/dl; p = 0.001). Similarly, the average HbA1c level was significantly higher in the blood of the 287T carriers as compared to 287M carriers: 7 ±1.87% vs. 6.3 ±1.15% (p = 0.020). The associations of FBG, 2HBG and HbA1c with Met287Thr polymorphism remained statistically significant after logarithmic transformation. The 287T carriers were more likely to have FBG ≥126 mg/dl (OR = 2.36) and 2HBG ≥200 mg/dl (OR = 2.86), although neither association was statistically significant. Notably, the average DMAsIII concentration was higher in urine of the 287T carriers as compared to 287M carriers before (p = 0.023) and after (p = 0.032) logarithmic transformation. Taken together, these results suggest that AS3MT/287T carriers are more likely to develop diabetes when exposed to iAs because they produce more DMAsIII than do 287M carriers.

PS 1981 METHYLATION OF ARSENIC BY RECOMBINANT HUMAN AS3MT/287M AND AS3MT/287T POLYMORPHS.

L. Ding[1], J. Saunders[1], Z. Drobná[1], D. J. Thomas[2] and M. Styblo[1]. *[1]Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC and [2]ETD, NHEERL, U.S. EPA, Research Triangle Park, NC.*

Arsenic (+3 oxidation state) methyltransferase (AS3MT) is the key enzyme in the pathway for methylation of inorganic arsenic (iAs). AS3MT polymorphism is, in part, responsible for inter-individual differences in iAs metabolism. AS3MT/M287T polymorphism that is found in ~10% of Caucasian and Hispanic populations has been shown to affect the profiles of iAs metabolites in urine of subjects chronically exposed to iAs. Previous work using a suboptimal in vitro system containing glutathione (GSH) showed a significant difference between the rates of iAs methylation by recombinant human AS3MT/287M and AS3MT/287T. We compared the catalytic properties of recombinant human AS3MT/287M and AS3MT/287T variants in an optimized in vitro reaction mixture containing arsenite (iAsIII), S-adenosylmethionine and an endogenous reducing system consisting of thioredoxin (Trx), TRx reductase (TR), and NADPH. The AS3MT variants methylated 0.1-1 μM iAsIII with similar efficiencies to yield primarily dimethylarsinate (DMAsV) and dimethylarsinite (DMAsIII); methylarsonate (MAsV) and methylarsonite (MAsIII) were only minor metabolites. However, MAsV and MAsIII yields increased in reaction mixtures containing 10 μM iAsIII. Addition of 1-10 mM GSH into the reaction mixture resulted in a 2- to 3-fold increase in the rate of iAs methylation by either variant, favoring production of trivalent methylated metabolites, MAsIII and DMAsIII. Under these conditions, the AS3MT/287T variant produced significantly more DMAsIII than did AS3MT/287M variant. Notably, in the absence of the TRx/TR/NADPH system, addition of GSH was not sufficient to support iAsIII methylation by either AS3MT/287T or AS3MT/287M. These results suggest that: (1) GSH is not essential for iAs methylation but is an important modulator of AS3MT activity in human tissues; (2) the carriers of AS3MT/287T genotype may be more susceptible to adverse effects of iAs exposure due to an increased production of DMAsIII. (This abstract does not reflect U.S. EPA policy.)

PS 1982 GST-T1 AND GST-M1 GENOTYPES MODULATE THE METABOLISM AND DIABETOGENIC EFFECTS OF INORGANIC ARSENIC.

Z. Drobna[1], L. Del Razo[2], G. Garcia-Vargas[3], D. Loomis[4] and M. Styblo[1]. *[1]Nutrition, CB#7461, UNC Chapel Hill, NC, Chapel Hill, NC, [2]Toxicology, CINVESTAV-IPN, Mexico City, Mexico, [3]Faculty of Medicine, Juarez University of Durango State, Durango, Mexico and [4]Epidemiology, University of Nebraska Medical Center, Omaha, NE.*

Glutathione S-transferase (GST)-T1 and GST-M1 are among enzymes involved in the metabolism and detoxification of inorganic arsenic (iAs). GST-T1 and GST-M1 null genotypes have been shown to affect the levels of methylated metabolites of iAs, methyl-arsenic (MAs) and dimethyl-arsenic (DMAs), in urine of subjects exposed to iAs. To streamline examination of GST-T1 and GST-M1 null genotypes in human DNA, we have developed a multiplex qPCR-High Resolution Melting procedure using the LightCycler 480 instrument and software. This procedure is based on analysis of the PCR product melting curve that represents the dynamics of thermal dissociation of double-stranded DNA. We used the newly developed procedure to identify GST-T1 and GST-M1 null genotypes in DNA samples collected from 238 residents of Zimapan and Lagunera (Mexico) who were exposed to iAs in drinking water. Null genotype for GST-T1 was detected in 17 (7.1%) subjects and for GST-M1 in 92 (38.7%) subjects; 5 individuals (2.1%) were carriers of both GST-T1 and -M1 null genotypes. Analysis of genotype-phenotype associations showed that GST-T1 null genotype is associated with a significantly higher urinary DMAs/MAs ratio: 7.21±5.46 as compared to 5.43±3.12 for GST-T1 positive subjects (p=0.022). GST-M1 null genotype did not affect urinary profiles of iAs metabolites. However, carriers of GST-M1 null genotype had, on average, significantly lower fasting blood glucose levels (81.22±18.31) than GST-M1 positive subjects (86.15±14.30, p = 0.0251). In summary, we have developed a high-throughput technique for identification of GST-T1 and GST-M1 null genotypes and validated it in a field study in Mexico. Our data suggest that the GST-T1 null carriers have a higher capacity for conversion of MAs to DMAs while GST-M1 null genotype may decrease diabetogenic effects of chronic exposures to iAs.

PS 1983 ARSENIC METHYLATION IS ASSOCIATED WITH BREAST CANCER RISK IN NORTHERN MEXICO.

M. E. Cebrián[1], A. Gandolfi[2], R. U. Hernandez[3], J. M. Ornelas[4], L. Torres-Sanchez[3] and L. Lopez-Carrillo[3]. *[1]Toxicology, Cinvestav IPN, Mexico City, DF, Mexico, [2]Pharmacology and Toxicology, University of Arizona, Tucson, AZ, [3]CISP, INSP, Cuernavaca, Morelos, Mexico and [4]Pathology, IMSS, Ciudad Obregón, Sonora, Mexico.*

Inorganic As exposure has been associated with skin, bladder, liver, lung, and prostate tumors in humans. However, most studies to date have not yet implicated As as a cofactor for breast cancer (BC). In northern Mexico, As exposure via drinking water is endemic and BC mortality is 3-fold higher than in the rest of the country. Our aim was to explore the potential association between As exposure, the profile of As metabolites and BC. From an ongoing population-based case–control study, the first 603 confirmed BC incident cases, as well as 477 population controls were available for this report. Women were directly interviewed about their reproductive and dietary history. The profile of As metabolites in urine was determined by HPLC/ICP-MS. Methylation capacity was assessed by calculating the percentage of arsenic species and the primary [MMA(V)/(As(III)+As(V)] and secondary [DMA(V)/MMA(V)] methylation indexes. The range of total urinary As values (TAs) was 0.4 to 254.9 ug/L. Urinary concentrations of Asi, DMA and TAs were slightly lower in BC cases. However, the percentage of MMA was significantly higher in BC cases, while the percentage of DMA was significantly lower. Age-adjusted odd ratios showed a significantly positive association between MMA/Asi values and BC risk (OR T3 vs T1 = 1.59; CI95=1.18-2.13; p for trend = 0.002). In contrast, the DMA/MMA ratio was negatively associated (OR T3 vs T1 = 0.48; CI95=0.35-0.65; p for trend = 0.000). This is the first report suggesting that As exposure may pose a risk for BC and that women with higher capacity to methylate in-

organic As to MMA(V) and a lower capacity to further methylate this metabolite to DMA(V), have a higher risk of BC. Further research is needed to investigate the role of genetic polymorphisms involved in As metabolism and their relevance for BC.
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PS 1984 INFLUENCE OF INDIGENOUS AMERICAN ANCESTRY AND BODY MASS INDEX ON ARSENIC METHYLATION EFFICIENCY IN NORTHWEST MEXICO.

P. Gomez-Rubio[1], M. M. Meza-Montenegro[2], E. Cantu-Soto[2], Y. C. Klimentidis[3] and W. T. Klimecki[1]. *[1]Pharmacology and Toxicology, University of Arizona, Tucson, AZ, [2]Environmental Sciences, Instituto Tecnologico de Sonora, Obregon, Sonora, Mexico and [3]Biostatistics, University of Alabama at Birmingham, Birmingham, AL.*

Environmental exposure to arsenic has been widely associated with the development of a vast array of cancerous and non-cancerous diseases. Human arsenic methylation efficiency, measured as the percentage of urinary monomethylarsonic acid excretion (%uMMA), has been reliably associated with the risk of arsenic-induced diseases. Previous epidemiological studies have suggested that Indigenous Americans may have better methylation efficiency than other ethnic groups. With the aim of characterizing the association between genetic Indigenous American ancestry (IA) and arsenic methylation efficiency, we studied a population of 635 arsenic-exposed individuals from Northwest Mexico. Arsenic species were determined in urine samples, anthropometric measurements were recorded, and a panel of 58 ancestry informative markers was used to determine the European and IA proportion in each individual. The mean urinary levels of inorganic arsenic, MMA and DMA were 26, 21.1 and 132.5 μg/L. Mean IA and body mass index (BMI) were 77% and 24.6 respectively. Adjusted multiple linear regression analysis (sex, age, AS3MT genetic variation, BMI, and total urinary arsenic) showed that IA proportion is significantly associated with arsenic methylation efficiency. Additionally we also observed a very strong association between BMI and arsenic methylation efficiency. Individuals with higher proportion of IA and higher BMI showed less %uMMA, and higher uDMA/uMMA than their counterparts. These associations highlight the importance of BMI and ancestry as potential arsenic-associated risk factors, and should be carefully considered in future arsenic association studies, especially those carried out in admixed populations. Additional studies will be needed in order to understand the mechanistic nature of these associations. (Funded by ES006694 and ES04940).

PS 1985 THE INFLUENCE OF GENETICS, NUTRITION, AND PREGNANCY ON ARSENIC METABOLISM: A LONGITUDINAL COHORT STUDY.

R. M. Gardner[1], K. Engström[2], K. Broberg[2] and M. Vahter[1]. *[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and [2]Department of Laboratory Medicine, Lund University, Lund, Sweden.*

Exposure to inorganic arsenic during pregnancy may negatively influence the offspring, although efficient metabolism of arsenic to dimethylarsinic acid (DMA) likely reduces the health risks. This study aimed to evaluate methylation of arsenic over the entire pregnancy and the influence of nutritional status and genetic background. We studied longitudinally the arsenic metabolite pattern in the urine of 294 pregnant women exposed to arsenic via drinking water and food in rural Bangladesh. Urine was collected from women at three time points throughout pregnancy (at approximately weeks 8, 19, and 30). Urinary arsenic metabolites were measured by HPLC-ICPMS. Women were genotyped for 22 polymorphisms in five methyltransferases: arsenic(+III)methyltransferase (AS3MT), DNA-methyltransferase 1a and 3b (DNMT1a and 3b), phosphatidylethanolamine transferase (PEMT) and betaine-homocysteine methyltransferase (BHMT). Because the data on arsenic metabolites in urine were collected longitudinally, changes in these biomarkers over time were modeled using linear mixed effects models fit with maximum likelihood estimation. Metabolism of arsenic to DMA increased markedly over the course of pregnancy, with the greatest improvement occurring in the first trimester, along with a marked decrease in the most risk-associated monomethylated metabolite. This improvement in methylation was not associated with nutritional status, including vitamin B12 and folate. Six AS3MT polymorphisms and four polymorphisms in the DNMTs significantly influenced metabolite pattern in the pregnant women, with consistent effects of genotype over the entire course of pregnancy. Efficient methylation to DMA was associated with improved urinary excretion of arsenic, relative to blood arsenic concentrations, indicating that micronutrient-independent up-regulation of arsenic metabolism occurring in early pregnancy may provide protection for the fetus.

PS 1986 DETECTION AND STABILITY OF METHYLATED TRIVALENT ARSENIC METABOLITES IN MOUSE LIVER HOMOGENATES.

J. Currier[1], J. Saunders[2], Z. Drobná[2] and M. Styblo[2]. *[1]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and [2]Nutrition, University of North Carolina, Chapel Hill, NC.*

Chronic exposure to inorganic arsenic (iAs) is associated with a variety of diseases, including cancer, hypertension and diabetes. Current evidence suggests that the toxic methylated trivalent metabolites of iAs, methylarsonite (MAsIII) and dimethylarsinite (DMAsIII), play a key role in the etiology of these diseases. Both MAsIII and DMAsIII have been detected in urine of subjects exposed to iAs. However, the rapid oxidation of DMAsIII and, to a lesser extent, MAsIII leads to difficulties in the analysis of these metabolites in samples of urine collected in field studies. Results of our previous work indicate that MAsIII and DMAsIII are relatively stable in a reducing cellular environment, suggesting that analysis of cells or tissues rather than urine, could be used to characterize internal exposures to these toxic metabolites. In the present study, we used the oxidation state-specific hydride generation-cryotrapping-atomic absorption spectroscopy (HG-CT-AAS) to examine presence and stability of MAsIII and DMAsIII in livers of mice exposed to iAs in drinking water. Liver homogenates were prepared in deionized water and stored at either 0°C or -80°C for up to 22 days. Tri- and pentavalent metabolites of iAs were analyzed in homogenates directly (without chemical digestion); analysis of homogenates digested in phosphoric acid was used to determine the recovery of As species during the direct analyses. In fresh homogenates, MAsIII and DMAsIII represented 12% and 45% of total As, respectively. Both MAsIII and DMAsIII were stable in homogenates stored at -80°C. In contrast, DMAsIII in homogenates stored at 0°C began to oxidize to its pentavalent counterpart after 1 day, whereas MAsIII remained stable for at least 3 weeks under these conditions. At least 95% of all As species were recovered by the direct analyses, suggesting that this method is suitable for quantitative analysis of iAs metabolites, including MAsIII and DMAsIII in tissues or cells collected in laboratory and population-based studies.

PS 1987 THE EFFECT OF PRENATAL FOLATE SUPPLEMENTATION ON DNA METHYLATION AND GENE EXPRESSION IN MALE CD1 MOUSE FETUSES EXPOSED *IN UTERO* TO ARSENIC.

V. Tsang[1], R. Fry[2], M. Niculescu[3], J. Saunders[1], M. Waalkes[4], M. Styblo[1] and Z. Drobna[1]. *[1]Nutrition, CB#7461, UNC Chapel Hill, NC, Chapel Hill, NC, [2]Environmental Sciences and Engineering, UNC Cahpel Hill, Chapel Hill, NC, [3]Nutrition Research Institute, Kannapolis, NC and [4]NTP at NIEHS, Research Triangle Park, NC.*

In humans and mice, inorganic arsenic (iAs), an environmental carcinogen, is methylated prior to excretion. Recent studies suggest that utilization of S-adenosylmethionine (SAM) for iAs methylation during prenatal exposure can cause cancer by changing fetal DNA methylation, followed by aberrant gene expression in adulthood. We examined whether prenatal folate supplementation would prevent iAs-induced changes in DNA methylation. Pregnant CD1 mice were exposed to 0 or 85 μg As/L of arsenite in drinking water and received a folate-supplemented or control diet from gestation day (GD) 8 to 18. At GD 18, fetal livers were harvested to determine concentrations of SAM and iAs metabolites. The methylation patterns for genomic DNA in the livers were examined using a two color Mouse CpG-Island Microarray containing 16,030 CpG islands. Molecular network analyses identified affected genes and associated networks. Expression of genes with modified methyl-CpG island profiles was examined by quantitative PCR. Treatments with iAs and/or folate increased SAM and S-adenosylhomocysteine concentrations in fetal livers as compared to untreated controls. Prenatal iAs exposure alone did not alter the DNA methylation status in mouse fetuses. In contrast, folate supplementation alone and, especially combined with iAs, altered the methylation of hundreds of CpG islands. The most affected genes were associated with the cell cycle, cancer, and neurological diseases. The exposure to iAs combined with folate supplementation altered the expression of cyclin dependent kinase inhibitors 1b and 2a, and of the imprinted tumor suppressor gene Dlk1. Our data suggest that when combined with exposure to iAs, prenatal folate supplementation results in extensive changes in fetal DNA methylation with possible consequences for fetal or offspring health.

PS 1988 AUTOPHAGY: A CELLULAR PROCESS STRONGLY ASSOCIATED WITH ARSENITE-INDUCED IMMUNOTOXICITY IN HUMAN LYMPHOBLASTOID CELL LINES.

A. M. Bolt, R. M. Douglas and W. T. Klimecki. *Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Inorganic arsenic is a global environmental toxicant that is associated with a diverse array of complex diseases, making it challenging to identify the mechanism underlying arsenic-induced cytotoxicity. A mounting body of literature from epidemiological and in vitro / in vivo studies has demonstrated that arsenic is a potent immunotoxicant, but the mechanism driving arsenic-induced immunotoxicity is not well established. We have previously demonstrated that in human lymphoblastoid cell lines (LCL), arsenic-induced cell death is strongly associated with the induction of autophagy. In this study we utilized genome-wide gene expression analysis and functional assays to characterize arsenic-induced immunotoxicity in seven LCL that were exposed to an environmentally relevant, minimally cytotoxic, concentration of sodium arsenite (0.75 uM) over an eight-day time course. Arsenite exposure resulted in inhibition of cell proliferation and the induction of autophagy (measured by expansion of acidic vesicles) over the eight-day exposure duration. Interestingly, there was a strong correlation between the extent to which arsenite exposure inhibited cell proliferation and the fold induction in autophagy in the different LCL analyzed. Gene expression analysis revealed that arsenite exposure globally increased lysosomal gene expression, which was associated with increased functional activity of the lysosome protease, cathepsin D. The lysosomal gene master regulator, transcription factor EB (TFEB) was also up regulated in arsenite exposed samples in a time-dependent manner, which could explain the concordant lysosomal gene expression. The arsenic-induced expansion of the lysosomal compartment in LCL represents a novel target that may offer mechanistic insight into the immunotoxic effects of arsenic. (Funded by ES 006694, ES 16652, and ES 04940)

PS 1989 DEVELOPMENTAL IMMUNOTOXICITY OF LOW DOSE ARSENIC EXPOSURE.

C. Kozul-Horvath[1], J. W. Hamilton[2] and R. Enelow[1]. [1]*Immunology, Dartmouth Medical School, Lebanon, NH and* [2]*Bay Paul Center for Comparative Molecular Biology & Evolution, Marine Biological Laboratory, Woods Hole, MA.*

Arsenic (As) exposure is a significant worldwide environmental health concern and chronic exposure via contaminated drinking water has been associated with an increased incidence of a number of diseases. We previously reported that As exposure has significant effects on immune responses to influenza infection in adult mice. In order to identify critical windows of developmental As exposure and specifically, the potential immunotoxic effects of such exposures at the current EPA standard of 10 ppb, C57B6 pups were exposed to 10 ppb As, either in utero or during the postnatal weaning period (via the dam). We observed that following in utero As exposure there was a decrease in productive matings. However birth outcomes, such as litter weight, size, and gestational length were unaffected. Following birth, the pups in both models of exposure had significant growth defects, which resolved following cessation of exposure. Additionally we observed significant As-associated alterations in immune cell populations within the lung and spleen, including T cells (CD4+ and CD8+) and B cells. These results suggest that low level As exposure via the mother can induce significant alterations in the growth of offspring and the development of the immune system, which may contribute to enhanced disease risk. (NIH-NIEHS SRP P42 ES007373)

PS 1990 ARSENIC ALTERS PURINERGIC TYPE 2 (P2) RECEPTOR Ca^{2+} SIGNALING ASSOCIATED WITH INNATE IMMUNITY IN HUMAN AIRWAY EPITHELIAL CELLS.

C. L. Sherwood[1, 2, 5], R. Lantz[2, 4, 5], J. L. Burgess[4, 6] and S. Boitano[1, 3, 4]. [1]*Respiratory Center, Arizona Health Sciences Center, Tucson, AZ,* [2]*Cell Biology and Anatomy, Arizona Health Sciences Center, Tucson, AZ,* [3]*Physiology, Arizona Health Sciences Center, Tucson, AZ,* [4]*SWEHSC, Arizona Health Sciences Center, Tucson, AZ,* [5]*Bio5 Institute, Arizona Health Sciences Center, Tucson, AZ and* [6]*College of Public Health, Arizona Health Sciences Center, Tucson, AZ.*

Arsenic is a natural contaminant of drinking water supplies worldwide. Consumption of arsenic-tainted water has been correlated with malignant and non-malignant lung diseases. Despite strong links with respiratory illness, underlying mechanisms of arsenic-induced disease remain unclear. Recent research on chronic lung disease has uncovered a key role for paracrine ATP signaling. We hypothesized that arsenic may elicit some of its detrimental effects on the airway through limitation of innate immune function, and specifically, through alteration of paracrine ATP and intracellular Ca^{2+} signaling in the airway epithelium. We examined the effects of acute (24 hr) exposure with environmentally relevant levels of arsenic (i.e., < 4 μM (~300 μg/L) as Na-arsenite) on wound-induced Ca^{2+} signaling pathways in an immortalized human bronchial epithelial cell line (16HBE14o-). We found arsenic reduces ATP-induced Ca^{2+} signaling in a dose-dependent manner and results in a reshaping of the Ca^{2+} signaling response upon wounding. We next examined arsenic effects on two ATP-mediated pathways for Ca^{2+} signaling: the metabotropic P2Y and ionotropic P2X receptors. Arsenic inhibited P2Y response to ATP; this included a transcriptional reduction of $P2Y_2$ following sub-micromolar (0.8 μM) or micromolar (3.9 μM) additions of arsenic. Arsenic induced a dose-dependent inhibition of P2X response to ATP; this included a transcriptional reduction of $P2X_4$ only at micromolar (3.9 μM) levels. Ingested arsenic rapidly reaches the airway epithelium where ATP signaling is essential in innate immune functions (e.g., ciliary beat, salt and water transport, bactericide production, and wound repair). Arsenic-induced compromise of such airway defense mechanisms may be an underlying contributor to chronic lung disease.

PS 1991 SODIUM ARSENITE AND HYPERTHERMIA ALTER EXPRESSION OF XPA, XPC, AND MSH2 IN RESPONSE TO CISPLATIN-INDUCED DNA DAMAGE AND INCREASE ACCUMULATION OF PLATINUM IN OVARIAN CANCER.

C. S. Muenyi[1], V. A. States[1], J. H. Masters[1], T. Fan[1, 2], W. Helm[1, 3] and J. States[1]. [1]*Pharmacology & Toxicology, University of Louisville, Louisville, KY,* [2]*Chemistry, Univesity of Louisville, Louisville, KY and* [3]*Obstetrics, Gynecology & Women's Health, Univesity of Louisville, Louisville, KY.*

Ovarian cancer (OC) is the leading cause of gynecologic cancer death in the USA. Recurrence rates are high after front-line therapy and most patients eventually die from platinum-resistant disease. Cisplatin resistance is associated with increased nucleotide excision repair (NER), decreased mismatch repair (MMR) and decreased platinum uptake. We hypothesize that sodium arsenite (NaAsO2) and hyperthermia will sensitize OC to cisplatin by altering DNA repair and increasing platinum accumulation. A2780 and A2780/CP70 OC cells were treated with cisplatin ± NaAsO2 at 37 or 39 oC for 1 h. Co-treatment with NaAsO2 and hyperthermia sensitized these cells to cisplatin. NaAsO2 ± hyperthermia decreased cisplatin-induced XPC, an NER protein. NaAsO2 and hyperthermia additively increased platinum accumulation in these cells. Nude mice were injected with A2780/CP70 cells to create intraperitoneal (IP) tumors. Expression of key NER (ERCC1, XPC and XPA) and MMR proteins (MSH2) were analyzed by western blot in metastatic tumors isolated from mice after hyperthermic IP chemotherapy (HIPEC). XPA induction by cisplatin was suppressed by hyperthermia (43 oC). NaAsO2 suppressed XPC induction by cisplatin. Hyperthermia ± NaAsO2 decreased cisplatin-induced XPC 24 h after perfusion. MSH2 levels were higher in tumors perfused with NaAsO2 plus cisplatin. NaAsO2 significantly increased initial accumulation of platinum in tumors, but had no effect on Pt retention. Hyperthermia had no effect on tumor Pt levels. Platinum and arsenic generally accumulated in systemic tissues during HIPEC and decreased 24 h after perfusion. Conclusion: NaAsO2 and hyperthermia have the potential to sensitize OC to cisplatin by inhibiting NER and increasing MMR and platinum accumulation. Supported in part by NIH grants P30ES014443 and R01ES011314; and NSF-EPSCoR grant EPS-0447479.

PS 1992 EPITHELIA MALIGNANTLY TRANSFORMED BY ARSENIC OR CADMIUM DRIVES NEARBY NORMAL STEM CELLS TOWARDS A MALIGNANT PHENOTYPE.

Y. Xu, E. Tokar and M. Waalkes. *National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Stem cells (SCs) likely play a key role in carcinogenesis and can be committed to differentiation pathways by various factors. We find that arsenic malignantly transforms the normal human prostate epithelial cell line (RWPE-1) into a malignant phenotype involving survival selection of SCs and overproduction of cancer SCs (CSCs). Cadmium (Cd) can also malignantly transform the same cells through mechanisms likely different from arsenic. Whether and how a malignant epithelium might affect nearby normal SCs is poorly defined. Thus, we studied the potential effects of neighboring arsenic- and Cd-transformed malignant prostate epithelia on the normal prostate SC line, WPE-stem cells, which was previously isolated from RWPE-1 cells. These SCs were exposed via transwells, which do not allow cell lines direct contact but allow soluble factors to interact between cells, to malignant transformants from chronic arsenic-exposed RWPE-1 cells (CAsE-PE cells) or to chronic Cd-exposed RWPE-1 cells (CTPE cells) or to normal RWPE-1 cells, all of which are isogenic. Matrix metalloproteinases (MMPs) play a crucial

role in invasion and metastasis of cancer cells and are markers of malignant transformation by arsenic or Cd. In SCs trans-cultured with CAsE-PE or CTPE cells, MMP transcript increased after only 1 week of trans-culture, and secreted MMP-2 and MMP-9 activity increased after 2 weeks. PTEN, a tumor suppressor gene with a critical role in SCs differentiation, is often inactivated in malignancies. PTEN transcript was highly suppressed after only 1 week of trans-culture of SCs with either CAsE-PE or CTPE cells. K5 and P63 are markers of prostate basal cells, the absence of which is used for pathological diagnosis of prostate cancer. K5 and P63 were also markedly suppressed by trans-culture of SCs with CAsE-PE or CTPE cells. Therefore, arsenic- and Cd-induced malignantly transformed prostate epithelia impacts nearby normal SCs, apparently driving them towards a malignant phenotype while not actually having physical contact.

PS 1993 OVERABUNDANCE OF PUTATIVE CANCER STEM CELLS IN HUMAN SKIN KERATINOCYTE CELLS MALIGNANTLY TRANSFORMED BY ARSENIC.

Y. Sun, E. Tokar and M. Waalkes. *National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Arsenic is a human skin carcinogen. Cancer is probably a disease driven by stem cells (SCs) and SCs are likely a key target during arsenic oncogenesis. In utero arsenic exposure predisposes mice to skin carcinoma formation with an overproduction of cancer SCs (CSCs) and distortion of CSC signaling and population dynamics. Therefore, here we hypothesized that accumulation of CSCs may occur in a human skin keratinocyte line during arsenic-induced malignant transformation in vitro. Thus, the HaCaT cell line, which was malignantly transformed by sodium arsenite (100 nM, 30 weeks; termed As-TM cells) in prior work, was further studied for the quantity and nature of SC/CSCs during transformation. SCs/CSCs were isolated from As-TM cells and control cells by using a positive magnetic bead isolation system that purifies CD34 positive cells. CD34 is a cell surface marker for both human skin SCs and CSCs. Using this system, there were 2.5 times more SCs/CSCs isolated from As-TM cells than from control cells. Holoclones are dense, tightly packed clusters of cells formed in culture by SCs/CSCs and enriched in these same cells. After isolation of SC/CSCs, holoclone production from CSCs isolated from As-TM cells was 2.5-fold higher at 1 week and 3.5-fold higher at 2 weeks than SCs isolated from control cells. SC characteristics and potential malignant phenotype were assessed in SC/CSCs isolated from control and As-TM cells and compared to their parental lines. Transcript level of SC/CSC markers were elevated in both As-TM CSCs and control SCs compared to parental cells, but CSCs from As-TM cells had elevated Oct-4, K5, K15 transcripts, and dramatically stronger staining for p63, Notch1, K19 compared to control SCs. As-TM CSCs also showed markedly elevated MMP-9 secretion and colony formation in agar, both indicators of cancer phenotype, even when compared to total population of As-TM cells. Thus, arsenic-induced malignant phenotype is particularly pronounced in SCs during transformation of human skin cells and occurs concurrently with an overproduction of CSCs.

PS 1994 TRANSFORMATION OF NORMAL HUMAN BREAST EPITHELIAL (MCF-10A) CELLS BY ARSENITE AND CADMIUM INDUCES THE OVER-EXPRESSION OF NEURONAL SPECIFIC ENOLASE (ENOLASE-2).

M. Soh, S. H. Garrett, C. Bathula, D. A. Sens, S. Somji and M. Sens. *Pathology, University of North Dakota, Grand Forks, ND.*

Arsenite (As^{+3}) and cadmium (Cd^{+2}) exposure have been associated with many types of cancers including bladder, skin, liver, prostate and lung, and in case of breast, they have been implicated in estrogen receptor mediated gene transcription, endocrine disruption, DNA damage and altered cell growth. Previous studies from our laboratory haves shown that both As^{+3} and Cd^{+2} can directly malignantly transform the non-tumorigenic urothelial cell line UROtsa. The goal of this study was to determine if both these heavy metals could also transform the breast epithelial cell line MCF-10A. For this purpose the MCF-10A cells were exposed to 1 μM As^{+3} or Cd^{+2} for a long period of time with the end point being the ability to form colonies in soft agar. It was the demonstrated that long term exposure resulted in selection of cells that were able to form colonies in soft agar. These transformed cells were subjected to microarray analysis and one of the differentially expressed gene that was identified was enolase-2 (ENO-2) which is a glycolytic enzyme that catalyses the conversion of 2-phosphoglycerate to phosphoenol pyruvate. ENO-2 has shown to be over expressed in neuronal and neuro-endocrine tumors. It is also over expressed in certain breast cancers that have a neuro-endocrine differentiation. The next goal of the study was to determine if Cd^{+2} and As^{+3} could induce the expression of this enzyme in the MCF-10A cells upon short and long term exposure. The results obtained indicate that both the metals can up-regulate the expression of ENO-2 in a time and dose dependent manner. Although the mechanism involved in the up-regulation of ENO-2 is unknown, our study provides evidence that the increased expression of ENO-2 may have implications in the increased glycolytic capacity of tumors

PS 1995 SPARC GENE EXPRESSION IS REPRESSED IN HUMAN UROTHELIAL CELLS (UROTSA) EXPOSED TO OR MALIGNANTLY TRANSFORMED BY CADMIUM OR ARSENITE.

J. L. Larson[1], S. Somji[2], D. A. Sens[2], M. Sens[2], S. H. Garrett[2] and J. R. Dunlevy[1]. [1]*Anatomy and Cell Biology, University of North Dakota, Grand Forks, ND and* [2]*Pathology, University of North Dakota, Grand Forks, ND.*

SPARC belongs to a class of extracellular matrix-associated proteins that have counteradhesive properties. The ability of SPARC to modulate cell-cell and cell-matrix interactions provides a strong rationale for studies designed to determine its expression in cancer. The objective of this study was to determine if SPARC expression was altered in cadmium (Cd^{+2}) and arsenite (As^{+3}) induced bladder cancer and if these alterations were present in archival specimens of human bladder cancer. The expression of SPARC was determined in human parental UROtsa cells, their Cd^{+2} and As^{+3} transformed counterparts and derived tumors, and in archival specimens of human bladder cancer using a combination of real time reverse transcriptase polymerase chain reaction, western blotting, immunofluoresence localization and immunohistochemical staining. It was demonstrated that SPARC expression was down-regulated in Cd^{+2} and As^{+3} transformed UROtsa cells. In addition, the malignant epithelial component of tumors derived from these cell lines were also down-regulated for SPARC expression, but the stromal cells recruited to these tumors was highly reactive for SPARC. This finding was shown to translate to specimens of human bladder cancer where tumor cells were SPARC negative, but stromal cells were positive. Acute exposure of UROtsa cells to both cadmium and arsenite reduced the expression of SPARC through a mechanism that did not involve changes in DNA methylation or histone acetylation. These studies suggest that environmental exposure to As^{+3} or Cd^{+2} can alter cell-cell and cell-matrix interactions in normal urothelial cells through a reduction in the expression of SPARC. The SPARC associated loss of cell-cell and cell-matrix contacts may participate in the multi-step process of bladder carcinogenesis.

PS 1996 DIFFERENCES IN THE EPIGENETIC REGULATION OF METALLOTHIONEIN-3 GENE EXPRESSION BETWEEN PARENTAL AND CADMIUM- OR ARSENITE-TRANSFORMED HUMAN UROTHELIAL CELLS.

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

Cadmium (Cd^{+2}) and arsenite (As^{+3}) and are human carcinogens and exposure to them has been associated with the development of bladder cancer. Previous work from our laboratory has shown that the UROtsa cell line can be directly malignantly transformed by both Cd^{+2} and As^{+3} and that the resulting tumors retain features of urothelial cancer. These transformed cell lines in cell culture do not express metallothionein-3 (MT-3); despite the fact that most human bladder tumors overexpress this gene. However, when they are transplanted as tumors they show strong expression of MT-3. This difference in MT-3 expression suggests that the mechanism of MT-3 gene silencing between the parental and transformed urothelial cells are different. The goal of this study was to determine if epigenetic modifications control urothelial MT-3 gene expression and if regulation is altered by malignant transformation by Cd^{+2} or As^{+3}. For this purpose, the parent and the transformed cell lines were exposed to the histone deacetylase inhibitor MS-275 and RNA was isolated and subjected to RT-PCR. The results obtained showed that MS-275 induced MT-3 mRNA expression in both parental UROtsa cells and their transformed counterparts. Chromatin Immunoprecipitation analysis showed that the metal-responsive transcription factor-1 (MTF-1) binding to the metal response elements (MRE) of the MT-3 promoter was restricted in parental cells under basal conditions, but MTF-1 binding to the MREs was unrestricted in the transformed cell lines. Histone acetylation of H4 and trimethylation of H3K4, H3K9 and H3K27 were compared between the parental and transformed cells. These studies showed that there is a fundamental difference in the accessibility of the MRE elements to MTF-1 binding within the MT-3 promoter between the parental UROtsa

cells and the transformed cells. This implicates histone modification as central to the differences in the regulation of MT-3 expression between normal urothelium and its transformed counterpart.

PS 1997 IL-8 OVER-EXPRESSION AND AUTOCRINE CELL ACTIVATION ARE KEY FACTORS IN MONOMETHYLARSENOUS ACID [MMA(III)]-INDUCED MALIGANT TRANSFORMATION OF HUMAN UROTHELIAL CELLS.

C. Escudero[1, 2], T. Wu[2], J. M. Camarillo[2] and A. Gandolfi[2]. [1]*Inmunologia y Biologia Celular y Molecular/CIEP, Universidad Autonoma de San Luis Potosi, SLP, Mexico and* [2]*Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

The association between chronic human exposure to arsenicals and bladder cancer development is well recognized; however the molecular mechanisms have not been fully determined. We previously demonstrated that chronic exposure to 50 nM of monomethyl arsenous acid [MMA(III)] leads to malignant transformation of an immortalized model of urothelial cells (UROtsa), with only 3 mo of exposure necessary to trigger the transformation-related changes. Those changes include the increase in cell proliferation rate, anchorage independent growth, and tumor formation after hetero-transplantation in SCID mice. In this phase, the cells over-express pro-inflammatory cytokines (IL-1β,IL-6,IL-8) consistent with the sustained activation of NFKb and AP1/c-jun, ERK2, and STAT3. IL-8 was over-expressed in our model 16 hr after exposure to MMA(III) and a sustained over-expression was observed in cells exposed for 3 mo. In this study IL-8 was also found increased in tumors produced from MMA(III) exposed cells after heterotransplantation in SCID mice. These treated UROtsa cells do express both receptors CXCR1 and CXCR2 suggesting that an autocrine cell activation could be important in cells transformation. Supporting this observation and consistent with IL-8 over expression, CXCR1 internalization was significantly increased after three mo of exposure to MMA(III). When unexposed UROtsa cells were exposed to rIL-8 the expression of MMP-9, cyclin D1, bcl-2, and VGEF was significantly increased; the same gene expression profile was found in cells exposed for 3 mo to MMA(III). After IL-8 was silenced in cells exposed for 3 mo to MMA(III) a decrease in cell proliferation rate was noted along with a decrease in anchorage-independent colony formation as well as a decrease in tumor progression related genes expression. These results suggest a key role of IL-8 in MMA(III)-induced UROtsa cells transformation.(NIEHS 04940).

PS 1998 INORGANIC ARSENIC CAUSES APOPTOSIS IN MOUSE CEREBRUM THROUGH AN OXIDATIVE STRESS-INDUCED MAPKS AND ER STRESS PATHWAY.

C. Huang[1], M. Lee[2], Y. Chen[3], T. Ho[1], C. Yen[4] and C. Su[5]. [1]*School of Chinese Medicine, China Medical University, Taichung, Taiwan,* [2]*Department of Surgery, Penghu Hospital, Department of Health, Executive Yuan, Penghu, Taiwan,* [3]*Department of Physiology, China Medical University, Taichung, Taiwan,* [4]*Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, Taiwan and* [5]*Department of Otorhinolaryngology, Head and Neck Surgery, Changhua Christian Hospital, Changhua, Taiwan.*

Arsenic pollution is a major public health problem throughout the world. Inorganic arsenic (iAs) is the predominant form and usually more harmful than organic ones, and increasing the risk of human diseases, such as peripheral vascular disease and cancer. However, the neurotoxic effect of iAs-induced is mostly unclear. Here, we investigated the toxic effect mechanisms of iAs (0.5 and 5 ppm, in drinking water), which was the possible exposed dose by ingestion in iAs-contaminated areas, in cerebrum of mice after administration for 6 consecutive weeks. iAs dose-dependently caused the increase of lipid peroxidation in plasma and cerebral cortex. iAs could also decrease the glutathione levels and NAD(P)H:quinone oxidoreductase-1 and glutathione peroxidase mRNA expression in the cerebral cortex. Antioxidant N-acetylcysteine effectively reversed iAs-induced oxidative stress damage in cerebrum. Moreover, iAs induced the activation of caspase-3, upregulation of Bax and Bak (pro-apoptotic) and downregulation of Mcl-1 (anti-apoptotic) mRNA expression in the cerebral cortex. Exposure to iAs could also trigger endoplasmic reticulum (ER) stress, as indicated by marked activating of glucose-regulated protein (GRP)-78, GRP-94, and C/EBP homologous protein (CHOP) mRNA. Moreover, western blot assay showed the increase of p38 activation and dephosphorylation of ERK1/2 were found in the cerebral cortex of iAs-exposed mice. These iAs-induced apoptosis-related signals could be significantly reversed by N-acetylcysteine. Therefore, our results suggest that iAs-induced oxidative stress cause apoptosis in cerebrum, and the signalings of p38 and ERK1/2-MAPKs and ER stress may be involved in iAs-induced neurotoxicity.

PS 1999 ALTERED EXPRESSION PROFILES OF microRNAs UPON ARSENIC EXPOSURE OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS.

Y. Wei[1], Y. Shi[2], X. Ma[3] and R. Li[2]. [1]*Department of Community Medicine, Mercer University School of Medicine, Macon, GA,* [2]*Key Laboratory of Pathobiology, Jilin University, Changchun, Jilin, China and* [3]*Department of Molecular and Cell Biology, Center for Systems Biology, University of Texas at Dallas, Richardson, TX.*

Exposure to high levels of inorganic arsenic that is present in drinking water of endemic regions has been shown to elicit various health effects. However, the molecular mechanisms of arsenic-induced vascular toxicity are not fully understood. In this study we analyzed microRNA expression profiles upon arsenic exposure of human umbilical vein endothelial cells (HUVECs). The cells were treated with 20 μM of sodium arsenite for 24 h in Iscove's Modified Dulbecco's Medium supplemented with 10% fetal bovine serum. Total RNA was purified with a Trizol reagent and the expression of microRNAs was examined by Exiqon miRCURY™ LNA microRNA chips. We found that 82 microRNAs were up-regulated and 60 were down-regulated by arsenic treatment as compared to the control group. The expression of some of the altered microRNAs was confirmed by real time RT-PCR. Analysis of cellular functions by using DAVID Bioinformatics Resources revealed that phosphoproteins and genes involved in alternative splicing are among the top categories targeted by both up- and down-regulated microRNAs upon arsenic treatment. A number of DNA motifs were identified in the promoters of the perturbed microRNAs upon arsenic treatment by promoter analysis using MEME software, though we were not able to associate these DNA motifs with known transcription factors. In conclusion, the results show that arsenic exposure of HUVECs could alter the expression profiles of microRNAs which target common cellular function categories, such as phosphoproteins and genes involved in alternative splicing. Our findings provide more insights on arsenic-induced vascular injury.

PS 2000 ARSENIC INDUCES EXPRESSION OF C-REACTIVE PROTEIN.

I. Druwe, J. J. Sollome, P. Sanchez-Soria, T. D. Camenisch and R. R. Vaillancourt. *Department of Pharmacology & Toxicology, The University of Arizona, Tucson, AZ.*

C-Reactive protein (CRP) is an acute phase protein in humans and elevated levels are produced in response to inflammatory cytokines that are associated with artherosclerosis, hypertension, cardiovascular disease and insulin resistance. Exposure to inorganic arsenic, a common and known environmental toxicant, also produces cardiovascular disorders and is associated with insulin-resistance. In a previous study, we showed that treatment of 3T3-L1 adipocytes with sub-cytotoxic concentrations of sodium arsenite (0.67 μM and 3 μM) resulted in an induction of a cytokine response. Upregulation of various interleukins included IL-2, IL-4, and IL-6. IL-6 is known to induce C-Reactive Protein expression. Here we show that treatment of HepG2 cells with sub-cytotoxic concentrations of sodium arsenite results in elevated CRP production and secretion. In addition, treatment of FvB mice with 100 ppb sodium arsenite in the drinking water for six months starting at weaning age (three weeks) resulted in dramatically higher levels of CRP as compared to control. Studies are on-going to investigate the ramifications that chronic elevated levels of CRP can have on both the cardiovascular system in mice and insulin-dependent signaling pathways in cells. This work was supported by ES004940 and 1F31ES016990-01A2.

PS 2001 INORGANIC ARSENITE IMPAIRS INSULIN-STIMULATED GLUCOSE UPTAKE IN 3T3-L1 ADIPOCYTES: INVOLVEMENT OF CELLULAR ADAPTIVE RESPONSE TO OXIDATIVE STRESS.

P. Xue[1, 2], Y. Hou[1, 2], J. Fu[1], C. G. Woods[1], H. Liu[1], Q. Zhang[1], G. Sun[2], M. E. Andersen[1] and J. Pi[1]. [1]*The Hamner Institutes, Reaserach Triangle Park, NC and* [2]*China Medical University, Shenyang, China.*

There is growing evidence that chronic exposure of humans to inorganic arsenic, a potent environmental oxidative stressor, is associated with the incidence of type 2 diabetes (T2D). One critical feature of T2D is insulin resistance in peripheral tissues, especially in mature adipocytes, the hallmark of which is decreased insulin-stimulated glucose uptake (ISGU). Despite the deleterious effects of reactive oxygen species (ROS), they have been recognized as a second messenger serving an intracellular signaling role for insulin signaling. Nuclear factor-erythroid 2–related factor 2 (NRF2) is a central transcription factor regulating cellular adaptive response to oxidative stress. This study proposes that in response to arsenic exposure, NRF2-mediated adaptive induction of endogenous antioxidant enzymes blunts insulin-stimulated ROS signaling and thus impairs ISGU. Exposure of differentiated

3T3-L1 cells to low-levels of arsenite (up to 2 μM for 1 week; non-cytotoxic) led to decreased ISGU in a dose- and time-dependent manner. The impaired ISGU was associated with increases in a battery of endogenous antioxidant enzymes that are regulated by NRF2. In addition, total antioxidant potential was significantly increased in the arsenite-treated cells. The increased antioxidant activity significantly inhibited net insulin-stimulated intracellular ROS accumulation and Akt phosphorylation. In addition, the effects of arsenite exposure on the expression of genes/proteins that are associated with classical insulin signaling pathway and inflammatory response were also evaluated in the adipocyte models. Taken together our studies suggest that low-level of arsenite triggers a cellular adaptive oxidative stress response to impair ROS signaling that is involved in ISGU, and thus causes adipocyte insulin resistance.

PS 2002 CHRONIC LOW-DOSE ARSENIC EXPOSURE ENHANCES HEPATIC INJURY INDUCED BY HIGH FAT DIET IN MICE.

M. Tan[1, 2], R. H. Schmidt[1, 2], H. Zhong[1, 2], J. States[1] and G. E. Arteel[1, 2]. *[1]Pharmacology and Toxicology, University of Louisville, Louisville, KY and [2]Alcohol Research Center, University of Louisville, Louisville, KY.*

Background: Arsenic is a ubiquitous contaminant in drinking water worldwide. Whereas arsenic exposure alone is known to cause liver injury, concentrations/doses of arsenic required to cause these diseases are generally higher than levels of arsenic present in the U.S. water supply. However, it is now well understood that physiological/biochemical changes that are alone pathologically inert can synergistically enhance the hepatotoxic response to a subsequent stimulus. Such a '2-hit' paradigm is best exemplified in chronic fatty liver diseases. Here, we test the hypothesis that low dose of arsenic exposure sensitizes liver to hepatotoxicity in a mouse model of non-alcoholic fatty liver disease. **Methods:** Male C57Bl/6J mice were exposed to low fat diet (13% calories as fat) or high fat diet (42% calories as fat) and tap water or arsenic (4.9 ppm as sodium arsenite in drinking water) for ten weeks. Body weight and food consumption were recorded. Plasma and histologic indices of liver damage (AST/ALT and histology), steatosis (Oil Red-O) and neutrophil infiltration (CAE) were determined. **Results:** High fat diet (± arsenic) significantly increased body weight gain in mice compared with low-fat controls. High fat diet caused significantly increased liver to body weight ratios; this variable was unaffected by arsenic exposure. High fat diet caused steatohepatitis, as indicated by histological assessment and by increases in plasma ALT and AST. Although arsenic exposure had no effect on these indices of liver damage in low-fat fed animals, it significantly increased the liver damage caused by high fat diet. **Conclusions:** These data indicate that arsenic exposure at doses that are not overtly hepatotoxic enhance the toxicity of high fat diet. These results also suggest that subhepatotoxic arsenic exposure might be a risk factor for the development of fatty liver disease in human populations at risk. (supported by NIEHS)

PS 2003 ARSENIC-IRON INTERACTIONS IN FERRITIN: A NEW MODEL FOR ARSENIC TOXICITY.

T. R. Radabaugh, T. J. Monks and S. S. Lau. *Southwest Environmental Health Sciences Center, Department of Pharmacology/Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.*

Arsenic (As) causes oxidative damage to a myriad of cellular targets via production of ROS. Although a number of models have been proposed to explain arsenic mediated ROS production, the mechanisms are not well understood. Ferritin is a 450kDa iron storage protein found throughout the body, but primarily in the liver. It consists of a hydrated ferric iron oxide-phosphate crystalline core surrounded by a protein shell. Ferritin stores free Fe and provides protection from Fe catalyzed ROS. To examine As-ferritin binding and its role in As toxicity, a series of *in vitro* experiments were performed. The data indicated that (i) arsenate, similar to its analog phosphate, binds ferritin only when Fe is present, (ii) arsenate binding is proportional to the amount of Fe in ferritin, (iii) arsenate inhibits the uptake of Fe by ferritin, and (iv) arsenate can compete with phosphate for binding sites. Of interest was the oxidation state of the ferritin bound arsenic, since the Fe(III) + As(III) → As(V) + Fe(II) redox reaction is well documented. We incubated [^{73}As]-As(III) with horse spleen ferritin in the presence of 5mM GSH. HPLC analysis of the ferritin bound As showed it to be >97% As(V). To test if As-ferritin interactions occurred *in vivo*, a rabbit was injected with [^{73}As]-arsenite and euthanized. Autoradiography and western analysis of rabbit liver cytosol confirmed that As binds ferritin *in vivo*. Overall the results suggest two things. 1) Ferritin may be a major As binding protein. This may explain why As is seen in high concentrations in the liver after acute challenge. 2) Our data suggest a model for As toxicity whereby intracellular arsenite is oxidized to arsenate and binds the Fe-oxide core of ferritin in a similar fashion to phosphate and other oxyanions. The resulting Fe(II) from this redox reaction is now free to produce ROS via the Fenton reaction. Because ferritin is found throughout the body, As-Fe redox reactions in ferritin may represent a major mechanism for the production of As-induced ROS. (P30ES006694)

PS 2004 REALGAR- AND CINNABAR- CONTAINING AN-GONG-NIU-HUANG WAN (AGNH) IS MUCH LESS ACUTELY TOXIC THAN SODIUM ARSENITE AND MERCURIC CHLORIDE.

Y. Lu[2], J. Yan[2], Q. Wu[2], J. Shi[3], J. Liu[1, 2] and J. Shi[2]. *[1]Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS, [2]Pharmacology, Zunyi Medical College, Zunyi, Guizhou, China and [3]Pharmacology, Guiyang Traditional Medicine College, Guiyang, Guizhou, China.*

An-Gong-Niu-Huang Wan (AGNH) is a famous traditional Chinese medicine used for brain emergencies. AGNH contains 10% realgar (As4S4) and 10% cinnabar (HgS). Both As and Hg are well-known for their toxic effects, and the safety of AGNH is of concern. To address this question, the acute toxicity of AGNH, realgar and cinnabar were compared to sodium arsenite (NaAsO2) and mercuric chloride (HgCl2). Mice were administrated orally AGNH at 1, 3 and 6 g/kg. AGNH at 3 g/kg contains 2.8 mmol As/kg as realgar and 1.18 mmol Hg/kg as cinnabar. Realgar, cinnabar, arsenite (0.28 mmol/kg, 10% of realgar) and HgCl2 (0.256 mmol/kg, 20% of cinnabar) were given orally to mice for comparison. Blood and tissues were collected 8 h later for toxicity evaluation. Serum alanine aminotransferase was increased by arsenite and blood urea nitrogen was increased by HgCl2. Total As accumulation after arsenite in liver (100-fold) and kidney (13-fold) was much higher than that after realgar. The accumulation of Hg after HgCl2 in liver was 400-fold higher and kidney 30-fold higher than after cinnabar. Histopathology showed moderate liver and kidney injuries after arsenite and HgCl2, but injuries were mild or absent after AGNH, realgar, and cinnabar. The expression of metallothionein-1, a biomarker of metal exposure, was increased 4-10 fold by arsenite and HgCl2, but was unchanged by AGNH, realgar and cinnabar. Thus, AGNH, realgar and cinnabar are much less toxic acutely than are arsenite and HgCl2. The chemical forms of As and Hg are extremely important factors in determining their disposition and toxicity.

PS 2005 CINNABAR-CONTAINING ZHU-SHA-AN-SHENG WAN IS MUCH LESS CHRONICALLY NEPHROTOXIC THAN MERCURY CHLORIDE AND METHYLMERCURY IN RATS.

J. Shi[2], F. Kang[2], Q. Wu[3], Y. Lu[3], J. Liu[1, 3] and J. Y. Kang[4]. *[1]Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS, [2]Pharmacology, Guiyang Traditional Medical College, Guiyang, Guizhou, China, [3]Pharmacology, Zunyi Medical College, Zunyi, Guizhou, China and [4]Medicine, University of Louisville, Lousville, KY.*

Cinnabar (contains 96% HgS) is widely used in traditional Chinese medicines. Mercury (Hg) is a toxic substance and the use of Hg-containing remedies is of concern. Zhu-Sha-An-Shen-Wan (ZSASW) contains 20% cinnabar, with Hg content (110 ppm) much higher than Food Chemical Codex (FCC)allowable limits for seafood (1 ppm), yet it is a popular non-prescription medicine in Chinese drug stores. To address its toxic potentials, ZSASW and cinnabar were compared with mercuric chloride (HgCl2) and methylmercury (MeHg) for nephrotoxicity. Adult Sprague-Dawley rats were orally administered with ZSASW (1.4 g/kg), cinnabar (0.2 g/kg), HgCl2 (0.02 g/kg), MeHg (0.001 g/kg), or saline for 60 days, and toxicity was determined. The exposure levels of Hg in ZSASW and cinnabar were 11-fold higher than in HgCl2 and 190-fold higher than in MeHg. Animal body-weight gain was decreased by HgCl2 (50%) and MeHg (-10%). Blood urea nitrogen and creatinine levels were increased by MeHg. Histology showed severe renal pathology following MeHg and HgCl2 treatments, but mild after ZSASW and cinnabar. Renal Hg contents were markedly increased in HgCl2 and MeHg groups but were not significantly elevated in ZSASW and cinnabar groups. Real-time RT-PCR revealed that the expression of kidney injury molecule-1 was increased 50-fold by MeHg, 4-fold by HgCl2, but was unaltered by ZSASW and cinnabar; the expression of matrilysin (MMP7) was increased 3-fold by MeHg. In contrast, the expression of N-cadherin was decreased by HgCl2. Thus, ZSASW and cinnabar are much less nephrotoxic than HgCl2 and MeHg, indicating that chemical forms of HgS, HgCl2, and MeHg are important in determining their disposition and toxicity.

PS 2006 REALGAR AND REALGAR-CONTAINING LIU-SHEN-WAN ARE LESS ACUTELY TOXIC THAN ARSENITE AND ARSENATE.

J. Liu[1, 2], S. Liang[2], Y. Lu[2], Q. Wu[2], J. Miao[2] and J. Shi[2]. [1]*Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS and* [2]*Pharmacology, Zunyi Medical College, Zunyi, Guizhou, China.*

Liu-Shen-Wan (LSW) is a widely-used traditional medicine containing realgar (As4S4). Arsenicals have been used as a remedy and a poison since ancient times. Arsenic (As) is a toxic metalloid, whether acute oral toxicity of realgar and LSW is comparable to inorganic arsenicals was investigated in mice. Mice were orally given LSW (60 and 200 mg/kg; 200 mg LSW contains 27 mg realgar), realgar (30 mg/kg, equivalent to 21 mg As/kg), and the equivalent As dose as sodium arsenite (NaAsO2), or as arsenate (Na2HAsO4). Acute toxicity and tissue As accumulation were determined 8 hrs later. Arsenite and arsenate increased serum alanine aminotransferase (ALT) levels, indicative of liver injury; Blood urea nitrogen (BUN) was also increased by arsenite and arsenate, indicative of nephrotoxicity. No elevations of ALT and BUN were observed in LSW and realgar groups. Histopathology showed more damage in arsenite- and arsenate-treated liver and kidneys, while in realgar- and LSW- treated animals, only mild alterations were seen. Hepatic and renal As contents were dramatically increased to 6200 and 3350 ng/g, respectively, after arsenite, but only increased to 260 and 180 ng/g after LSW. The expressions of arsenic-sensitive stress genes, namely metallothionein-1 and heme oxygenase-1, were increased after arsenite or arsenate by 3-10 folds, but were unaltered after LWS and realgar. Realgar and LSW are much less toxic than arsenite and arenate. The use of total As content to evaluate the safety of realgar-containing traditional medicines is not scientifically sound.

PS 2007 AQUAGLYCEROPORIN 3 IS AN ARSENIC TRANSPORTER IN THE ATLANTIC KILLIFISH (*FUNDULUS HETEROCLITUS*): EFFECTS OF SALINITY.

D. Jung[1], B. Jackson[1], B. MacIver[2], C. Chapline[3], D. Sato[3], J. R. Shaw[3, 4] and B. A. Stanton[1, 3]. [1]*Dartmouth Medical School, Hanover, NH,* [2]*Beth Israel Deaconess Medical Center and Havard Medical School, Boston, MA,* [3]*Mt. Desert Island Biological Laboratory, Salisbury Cove, ME and* [4]*Indiana University, Bloomington, IN.*

Aquaglyceroporins (AQP3, 7, 9 and 10) are membrane proteins that transport water in a wide variety of cells. Previous studies have also shown that aquaglyceroporins mediate the cellular uptake of arsenic. To begin to elucidate the role of aquaglyceroporins in mediating arsenic uptake in the environmental model species, *Fundulus heteroclitus* (Atlantic killifish) we cloned kfAQP3 and measured arsenic transport into HEK293T cells expressing kfAQP3 and exposed to 2ppb arsenic (sodium arsenite). Compared to control cells, HEK293T cells expressing kfAQP3 accumulated significantly more arsenic in a time dependent manner. In addition, expression of kfAQP3 in Xenopus oocytes significantly enhanced water, glycerol and urea uptake. Thus, like other AQP3s, kfAPQ3s transport water, glycerol, urea and arsenic across cells membranes. . In rat kidney cells an increase in osmolality increases APQ3 expression, thus, we conducted studies to determine if transfer from fresh water to seawater alters kfAQP3 gene expression. An increase in the osmolality of swim water increased plasma osmolality and cortisol levels (Shaw et al. 2007) but reduced kfAQP3 gene expression by 95.4 ± 1.6 % ($p<0.001$). In summary, these studies demonstrate that kfAQP3 mediates the cellular uptake of arsenic and because seawater down-regulates kfAQP3 gene expression, predicts that the uptake of arsenic from swim water will be significantly greater in fresh water acclimated fish than in sea water acclimated fish.

PS 2008 LOW-LEVEL ARSENITE EXPOSURE THROUGH DRINKING WATER INCREASES BLOOD PRESSURE AND PROMOTES CONCENTRIC LEFT VENTRICULAR HYPERTROPHY IN MICE.

P. Sanchez Soria[1], D. Broka[1] and T. D. Camenisch[1, 2, 3]. [1]*Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ,* [2]*Steele Children's Research Center, University of Arizona, Tucson, AZ and* [3]*Southwest Environmental Health Sciences Center, University of Arizona, Tucson, AZ.* Sponsor: J. Gandolfi.

High incidence of cardiovascular diseases has been well studied and linked to populations with elevated arsenic content in their drinking water. Although this correlation has been well studied in many epidemiological studies, a lack of experimental models to study mechanisms of arsenic-related cardiovascular pathogenesis has limited our understanding of how arsenic may be an important underlying cause for the development of hypertension and left ventricular hypertrophy. Our studies show that mice chronically exposed to 100 ppb sodium arsenite for 6 months through drinking water show an increase in both systolic and diastolic blood pressures. An additional group exposed to 50 ppb has been added to our study, and data is currently under analysis. Echocardiographic studies as well as histological sections show concentric left ventricular hypertrophy. Echocardiography imaging in M-mode shows a 52 % increase in left ventricular mass in arsenic treated animals, when compared to controls, with no significant changes in stroke volume. These results are consistent with the phenotypic changes in hearts showing concentric left ventricular hypertrophy, which results in increased peripheral resistance with no change in stroke volume. We also see increased ventricular myocardial thickness in mice exposed to arsenic in-utero, in conjunction with structural abnormalities in valve morphology, suggesting that arsenic may play an important role in the developmental basis of adult cardiac disease. Thus arsenic exposure might be an important contributor to cardiovascular disease onset by affecting cardiac morphology and function. (NIH ES 04940; ES06694; SWEHSC P30ES006694)

PS 2009 RELATIVE BIOAVAILABILITY OF ARSENIC CONTAMINATED SOILS IN A MOUSE MODEL.

M. F. Hughes[1], K. D. Bradham[2], K. G. Scheckel[3], P. Seales[1], G. Lee[1], C. Nelson[2], A. Yeow[4], S. Harper[2] and D. J. Thomas[1]. [1]*ORD/NHEERL, U.S. EPA, Research Triangle Park, NC,* [2]*ORD/NERL, U.S. EPA, Research Triangle Park, NC,* [3]*ORD/NRMRL, U.S. EPA, Cincinnati, OH and* [4]*SAB, U.S. EPA, Washington, DC.*

Exposure to As contaminated soils compels extensive soil cleanups so that human health risks are minimized. To improve exposure estimates and potentially reduce remediation costs, determination of the bioavailability of As in soils is needed. Traditional in vivo models for arsenic bioavailability (i.e. juvenile swine and non-human primates) are costly and time-consuming.The objective of this study was to develop an in vivo mouse model to assess As bioavailability in soils. The test soils were from residential sites impacted by former smelter and mining activities. Total As concentrations, measured by Instrumental Neutron Activation Analysis (INAA) in soils ranged from 173 to 6899 mg As/kg. Female C57BL/6 mice (4 to 6 wks) were housed in groups of 3 in metabolism cages and provided ad libitum tap water and either AIN-93G purified rodent diet, amended diets containing one of nine test soils (1% w/w soil:diet), amended diets containing one of two As reference soils, or a sodium arsenate-amended (7 mg As/kg) diet. Mice were exposed to one of the diets for 9 days. Feed consumption was measured daily. Urine was collected daily. On the tenth day, the animals were humanely euthanized. Urine was pooled for each cage and analyzed for As by INAA. Absolute bioavailability (ABA) ([cumulative urinary As/cumulative As consumed in feed]*100) was about 60% for the sodium arsenate amended diet and ranged from 7-32% in the test diets. The ABA of the two As reference soils was about 26%. Relative bioavailability (RBA) ([ABA test diet/ABA sodium arsenate diet]*100) of the test soils ranged from 11 to 53%. The RBA of the two As reference soils was about 43%. The development of a mouse model to assess As bioavailability can provide reliable data that can be obtained quickly, at a reasonable cost and improve exposure and risk assessment. (This abstract does not represent US EPA policy.)

PS 2010 EVALUATION OF A COMBINATION OF AN ISOFLAVONE AND AN ACE INHIBITOR FOR PREVENTION OF RADIATION-INDUCED INJURY.

M. R. Landauer[1], T. A. Davis[2] and R. M. Day[3]. [1]*Armed Forces Radiobiology Research Institute, Bethesda, MD,* [2]*Department of Regenerative Medicine, Naval Medical Research Center, Silver Spring, MD and* [3]*Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD.*

We previously demonstrated in mice that genistein prevents lethality from gamma irradiation by protecting hematopoietic stem cells. Here we evaluated genistein in combination with the angiotensin converting enzyme (ACE) inhibitor, captopril, for reduction of radiation-induced hematopoietic injury. We measured the effects of genistein and captopril alone and in combination on survival, blood cell recovery, micronuclei, and repopulation of hematopoietic progenitor cells. In C57BL/6J mice, 8.25 Gy cobalt-60 total body irradiation (TBI) results in 0% survival after 30 days. Administration of captopril in the drinking water, from 1 hr through 30 days postirradiation, increased survival to 55%. One subcutaneous injection of genistein administered 24 hr before TBI resulted in 72% survival. Genistein + captopril increased survival to 95%. Enhanced survival was reflected in improved recovery of red blood cells, total bone marrow cells, and splenocytes. The drug combination enhanced early recovery of erythroid progenitors (CFU-E and BFU-E) and myeloid progenitors (CFU-GM). Genistein protected cells from radiation-induced DNA damage, but captopril had no effect. These data suggest that genistein and captopril protect the hematopoietic system from radiation injury via different mechanisms. This unique combination of two nontoxic, well tolerated compounds provides a novel approach to radiation protection.

PS 2011 IDENTIFICATION OF A NOVEL VX METABOLITE FROM MINIPIG LIVER MICROSOMES *VIA* LC-Q-TOF MASS SPECTROMETRY.

J. M. McGuire[1], M. E. Parrish[2], E. M. Jakubowski[1] and S. A. Thomson[1]. [1]*U.S. Army Edgewood CB Center, Aberdeen Proving Ground, MD and* [2]*SAIC, Gunpowder, MD.*

VX(O-Ethyl-S-[2-(diisopropylamino)ethyl] methylphosphonothioate is an extremely toxic organophosphate chemical warfare nerve agent (CWNA) that rapidly inhibits acetylcholinesterase. While the use of CWNAs is forbidden by an international convention, nerve agents have been stockpiled in numerous countries and remain a threat to both civilian and military personnel. The biochemical reactions at the tissue and organism levels induced by CWNAs are very complex. If metabolomic work can identify key metabolites present in a given condition, this information can more adequately describe mechanisms of toxicity and potential biochemical points for developing effective CWNA countermeasures. To that end, we have been examining the metablolites formed following incubation of Göttingen Minipig liver microsomes with VX. Although previous studies have been done with various liver tissue homogenates from several animal species, we have focused on isolating individual functional networks, such as the cytochrome P450 (CYP) superfamily of oxidative hemoprotein enzymes. Activating the CYP enzymes with NADPH cofactor produced a novel metabolite of VX,(O-Ethyl-S-[2-(isopropylamino)ethyl] methylphosphonothioate, which was identified using liquid chromatography coupled with quadrupole/time-of-flight mass spectrometry. Although this mono-isopropyl derviative of VX has been previously identified in VX-ozone and UV irradiation reaction mixtures, to our knowledge this is the first time this compound has been identified in a biochemical reaction.

PS 2012 COMPARISON OF THE EFFECT UPON AND RECOVERY OF ACETYLCHOLINESTERASE (ACHE) ACTIVITY IN VARIOUS TISSUES AND BLOOD AFTER SUB-LETHAL INHALATION AND SUBCUTANEOUS CYCLOSARIN (GF) EXPOSURES.

C. E. Whalley[1], L. A. Lumley[2], J. O'Donnell[2], D. Miller[1], W. Muse[1] and T. Shih[2]. [1]*ECBC, Aberdeen Proving Ground, MD and* [2]*USAMRICD, Aberdeen Proving Ground, MD .*

AChE activity data was collected following either subcutaneous (SC) or whole body inhalation (IH) exposures using two different sub-lethal GF doses (0.1 or 0.4 x LD50/LCt50) in male Hartley guinea pigs from various tissues and blood at sequential time points. Following the higher IH GF exposure, blood AChE was 84% depressed and at 48hr was 60% depressed; 7 days later blood AChE was still 49% depressed. A similar profile occurred with the lower IH exposure. With both SC exposure concentrations AChE was also depressed but the AChE recovery process began much earlier (24-48hr). AChE activity tissue results: Following IH exposure, eye, lung, heart, kidney and liver activity was significantly depressed (time intervals varied) but brain (prefrontal cortex/striatum) and diaphragm activity was essentially unaffected; following SC exposure, brain, eye, lung, heart, diaphragm, and kidney activity was significantly depressed (effective GF exposure concentration and time intervals varied) while liver activity was essentially unaltered. In conclusion, sub-lethal IH and SC GF exposures depressed blood AChE activity substantially with slow recovery. Blood AChE recovery was not totally complete 48-72hr after either SC or IH exposures. With tissue AChE activity, both exposure routes and both GF concentrations caused significant reductions in AChE activity in eye, lung and heart. Both IH exposure concentrations significantly inhibited activity in kidney and liver but not in brain or diaphragm. In contrast, only the higher SC GF exposure significantly inhibited AChE activity in these four tissues. Recovery time for tissue AChE activity varied somewhat, 24-168hr (IH exposure) and from ~4hr to >48hr (SC exposure). Data will be utilized to improve physiologically based pharmacokinetic-pharmacodynamic models. Information generated by this study is extremely relevant to force health protection concerns that also include individual protection, decontamination and environmental surveillance.

PS 2013 A DEEP DERMAL SKIN INJURY FOLLOWING EXPOSURE OF HAIRLESS GUINEA PIGS TO SULFUR MUSTARD VAPOR.

S. Dachir, M. Cohen, L. Tverya, R. Sahar, H. Gutman, R. Gez, R. Brandeis, V. Horwitz and T. Kadar. *Pharmacology, Israel Institute for Biological Research, Ness Ziona, Israel.* Sponsor: J. Graham.

Skin injuries following exposure to sulfur mustard (HD) are characterized by various symptoms including inflammatory response, vesications, necrosis and ulcerations of the exposed skin that are dose and exposure duration dependent. Although HD was first introduced during WW1, no effective treatment is yet available, and very often HD-induced injuries result in long-term complications. In order to develop a treatment that will improve the healing process and prevent skin damage and long-term effects, an experimental model of a deep dermal skin injury following exposure to HD was established and characterized in hairless guinea pigs (HGP). Clinical evaluation was conducted using reflectance colorimetry, trans-epidermal water loss and wound area measurements. Analysis of prostaglandin E2 (PGE2) content and metalloproteinase-2 & 9 (MMP) activity, as well as histopathological and immunohistochemical assessment of the extent of the injury were conducted up to four weeks post-exposure. Typical symptoms of HD skin injury developed including erythema and edema, impairment of skin barrier and severe ulcers. PGE2 content and MMP activity were elevated during the wound development and during the healing process. Histological evaluation revealed severe damage to the epithelium and deep dermis and vesications. At four weeks after exposure healing was not completed: significantly impaired stratum corneum, inflammatory cells, hemorrhages, absence of hair follicles and epithelial hyperplasia were observed. These results confirm the use of the deep dermal skin injury in the HGP as a suitable model that can further be utilized for the investigation of the pathological processes of acute as well as long term injuries and for testing treatments efficacy. This work was supported by the U.S. Army Medical Research and Material Command under Award#: W81XWH-08-2-0128.

PS 2014 REACTIVATION OF ORGANOPHOSPHATE INHIBITED ACETYLCHOLINESTERASE (ACHE) BY NOVEL PYRIDINIUM OXIMES IN THE CENTRAL NERVOUS SYSTEM OF RATS.

E. C. Meek[1], H. W. Chambers[1], J. Gearhart[2], R. B. Pringle[1] and J. E. Chambers[1]. [1]*Center for Environmental Health Sciences, Mississippi State University, Mississippi State, MS and* [2]*AFRL, Wright-Patterson AFB, OH.*

Organophosphate (OP), e.g. nerve agent, exposure resulting in the inhibition of AChE with subsequent overstimulation of the nervous system, may result in neurological deficits and death. Traditional therapies for OP exposure include atropine and an oxime (2-PAM) to reactivate the OP inhibited AChE. A weakness of the currently approved oximes is their limited ability to cross the blood-brain barrier (BBB). A series of novel pyridinium oximes has been synthesized to incorporate moieties increasing BBB penetration. Oximes were evaluated in vitro for their abilities to reactivate AChE inhibited by nerve agent surrogates, a sarin surrogate, phthalimidyl isopropyl methylphosphonate (PIMP), or a VX surrogate, nitrophenyl ethyl methylphosphonate (NEMP), which leave AChE phosphorylated with the same moieties as sarin or VX, respectively. Rat brain homogenate was incubated with PIMP or NEMP yielding about 80% AChE inhibition, followed by an oxime 0.1mM, and AChE activity measured. In vitro reactivation varied among oximes but was similar for surrogates, ranging from 21-82% for PIMP and 29-76% for NEMP, with 2-PAM yielding about 90%. Oxime lipophilicities (n-octanol/water partition coefficients) ranged from 0.009 to 2.244 and were greater than 2-PAM (0.006). A subset of oximes that demonstrated in vitro reactivation greater than 40% was selected for testing in vivo. Rats were administered 0.325mg/kg ip of a sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP) or 0.4mg/kg of NEMP yielding 70-80% brain AChE inhibition, followed by im administration of 2-PAM or novel oxime (0.1mmole/kg) at the time of peak brain AChE inhibition. Ten of the 16 novel oximes tested yielded 10-35% brain AChE reactivation, indicating their ability to cross the BBB and reactivate OP inhibited brain AChE, demonstrating their therapeutic potential for exposure to nerve agents. Supported by Defense Threat Reduction Agency: 1.E0056_08_AHB_C

PS 2015 CATECHOLAMINE OVERLOAD AND CARDIAC LESION FORMATION IN RATS FOLLOWING EXPOSURE TO THE NERVE AGENT SOMAN.

J. H. McDonough, K. E. Van Shura, M. E. Lyman, B. A. Capacio, J. C. O'Donnell and K. A. Whitten. *U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

Nerve agents are potent inhibitors of the enzyme acetylcholinesterase. The resulting cholinergic over-stimulation causes respiratory distress, convulsions, seizures, and eventually death. Several studies have shown that nerve agent exposure can result in cardiac damage. We investigated if this cardiac damage may be due to nerve agent-induced excessive release of catecholamines. We first determined the incidence of cardiac lesions 24 hr following administration of 0.6, 0.8, 1.0, or 1.2 X LD50 of the nerve agent soman or saline to different groups of rats. Only animals in the 1.0

and 1.2 LD50 exposure groups displayed robust signs of nerve agent intoxication and also had cardiac lesions; animals at the 0.6 or 0.8 X LD50 doses showed mild or no signs of intoxication and were free of cardiac damage. Next, rats were challenged with 1.2 X LD50 soman, and different groups of animals were euthanized at progressively longer delays following agent challenge. Plasma obtained at the time of euthanasia was assayed for norepinephrine, epinephrine and troponin, and the heart tissue was prepared with Masson's trichrome to detect cardiac lesions. The results showed that 15-30 min after nerve agent exposure there were significant increases in epinephrine and norepinephrine levels, but no changes in cardiac troponin levels. At 2-4 hr after intoxication, cardiac troponin levels increased and elevations persisted at least 24 hr after exposure. The differential timing of the catecholamine and troponin increases supports the hypothesis that the release of catecholamines triggers the subsequent cardiac damage. This may result from a direct overstimulation of the sympathetic nervous system by the high levels of acetylcholine. The elicitation of convulsive activity by the nerve agent may be contributory, since only animals that developed pronounced convulsions developed cardiac lesions, whereas animals that displayed no or only minor signs of intoxication all had normal cardiac tissue.

PS 2016 CENTRALLY ACTING THERAPEUTIC ADJUNCTS FOR NERVE AGENT INTOXICATION.

I. Koplovitz*, J. H. McDonough and T. Shih. *Research Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

The brain is a major target for the toxic action of organophosphorus nerve agents (NA). Two therapeutic approaches were conducted to assess the ability of centrally active therapies to improve treatment outcomes following lethal NA intoxication. One approach was to investigate tertiary oxime (MINA) that can readily penetrate into the brain to determine whether reactivation of central AChE inhibited by NA will increase survival and terminate seizures. The other approach was to evaluate the survival benefit of adding scopolamine (SCP) to current treatment regimens. All studies were conducted in male Hartley guinea pigs exposed s.c. to lethal and seizure-inducing doses of various NA. In reactivation studies, the tertiary oxime was administered 15 min after 1 x LD50 of sarin, soman, cyclosarin, or VR; peripheral tissue, blood and regional brain oxime dose-dependent AChE activity was determined 45 min later. Oxime doses from these studies were used as treatments 1 min after NA with or without atropine (ATS) and/or 2-PAM to assess the influence on 24-hr survival. Various oxime doses were evaluated for antizeizure activity when administered at the time of and up to 40 min after seizure onset. SCP studies were conducted to determine its survival benefit as an adjunct to ATS plus quaternary oxime (2-PAM or MMB-4) treatment administered 1 min after 2 x LD50 of various NAs with or without pyridostigmine (PB) pretreatment. Human relevant doses of SCP, ATS, 2-PAM, and PB were evaluated. Tertiary oxime resulted in dose-dependent increases in AChE activity in the brain, improved survival and terminated seizures. Seizures could be terminated even when the tertiary oxime was administered 40 min after seizure onset. SCP increased survival at 24 hr, which appeared to be dependent on recovery/restoration of AChE activity either through oxime reactivation or decarbamlyation of PB-protected AChE. Both centrally acting oxime and SCP appear to be viable therapeutic approaches as adjunct treatments to mitigate the toxic effects of NA and improve treatment outcomes.

PS 2017 RICIN TOXICITY IN BALB/C 3T3 CELLS: DOSE DEPENDENT PROTEIN EXPRESSION DETERMINED BY MASS SPECTROMETRY BASED PROTEOMICS.

V. H. Bevilacqua[1], S. Deshpande[2], J. S. Madren-Whalley[3], R. Jabbour[4], K. Jones[5], L. M. Reilly[6] and J. S. Rice[7]. *[1]Point Detection Branch, Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, [2]STC, Edgewood, MD, [3]Biotechnology Branch, Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, [4]SAIC, Gunpowder, MD, [5]NuSep, Bogart, GA, [6]Physical Science Department, Bethany College, Bethany, WV and [7]Elona Biotechnologies, Inc., Greenwood, IN.* Sponsor: J. Sekowski.

Ricin has remained a significant potential biological threat agent due in part to its wide availability and ease of extraction from the castor bean. It is a glycoprotein made up of A- and B-chains with the B-chain facilitating transport of the lectin into the cell. Once inside, it kills the cell by inhibiting protein synthesis. Ricin is considered extremely toxic to man by multiple routes of exposure. Proteomics based on mass spectrometry (MS) data can yield biomarker and relative protein expression information for interrogation of physiological changes in toxin-dosed cells. We have analyzed trypsinized cellular protein extracts from BALB/c 3T3 murine fibroblasts dosed with three concentrations of ricin (IC_{20}, IC_{50}, IC_{80}) and a vehicle control by liquid chromatography tandem MS. Nine hundred proteins were characterized employing bioinformatics methods including in-house (ABOID, Edgewood Chemical Biological Center) and external (ProteoIQ, NuSep) software. Approximately 5% of the observed proteins were common to all dose levels, indicating that ricin did not affect relative expression of these proteins, while more than 10% of observed proteins were unique to each specific dose level. These results indicate a dose-dependent response of relative protein expression to ricin exposure by 3T3 cells. Ongoing pathway analysis may reveal the survival mechanism for cells dosed at low levels, which in turn will provide insight into the related 3T3 physiological changes required to overcome ricin dosing. Funding: The Edgewood Chemical Biological Center In-House Laboratory Independent Research Program.

PS 2018 ASSESSMENT OF PRO-2-PRALIDOXIME (PRO-2-PAM) THERAPY FOR EXPOSURE TO ORGANOPHOSPHATE AGENTS IN GUINEA PIGS.

J. C. DeMar[1], S. M. Somerville[1], R. H. Ratcliffe[1], T. C. Ku[1], N. M. Nur[1], B. M. Ursic[1], S. M. Schulz[2], K. H. Smith[2], E. D. Clarkson[2] and R. K. Gordon[1]. *[1]Regulated Laboratories, Walter Reed Army Institute of Research, Silver Spring, MD and [2]Medical Toxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

Novel therapeutics to overcome neuro-toxic effects of organophosphate (OP) agents are needed due to past use in combat and terrorism. Standard exposure therapy includes pralidoxime chloride (2-PAM), which restores OP-inhibited PNS acetylcholinesterase (AChE) activity. Since 2-PAM is charged, it cannot cross into the brain to restore CNS AChE. In guinea pigs, we evaluated the ability of pro-2-PAM, a non-polar pro-drug of 2-PAM, to protect the brain after OP-agent exposure. First, animals were pre-treated with pyridostigmine bromide and then subcutaneously given 1 × LD_{50} of the OP-agent diisopropylfluorophosphate (DFP) and treated with pro-2-PAM and PNS acting atropine methyl bromide. Seizure (by EEG radiotelemetry) was alleviated, brain AChE activity (by enzyme assay) was increased, and hippocampal cell death (by histopathology at 24 h post-exposure) was prevented. In contrast, 2-PAM did not afford this protection. Pro-2-PAM reduced DFP-induced brain damage with treatment delays up to 60 min and at recoveries to 7 days. Next, we evaluated pro-2-PAM therapy to the chemical warfare OP-agents soman (GD), VR, and VX. In pyridostigmine pre-treated animals cutaneously exposed to GD, VR, or VX and treated with pro-2-PAM and CNS active atropine sulfate, there was reduced hippocampal damage to 10, 3, and 3 × LD_{50}, respectively, at 24 h. Brain AChE activity also increased compared to exposed non-treated controls. 2-PAM, however, did not show similar CNS-protection. In conclusion, pro-2-PAM is a promising therapeutic for treating the detrimental effects of OP-agent poisoning in the brain.
Support: CounterACT NINDS U01 NS058166-01 and DTRA 1.E0033_07_WR_C. Opinions or assertions contained herein are private views of the authors, and are not to be construed as official or as reflecting views of the NIH, US Army, or DoD.

PS 2019 SULFUR MUSTARD-INDUCED RESPONSES OF AIRWAY AND SKIN *IN VITRO.*

J. Seagrave, W. Weber and G. Grotendorst. *Lovelace Respiratory Research Institute, Albuquerque, NM.*

Sulfur mustard (SM) is a vesicant that has been used in warfare and is a potential threat to civilians either from acts of terrorism or through accidental exposure to abandoned military caches of the chemical. Exposure of the skin produces blisters and slow-healing wounds resulting in scarring, while exposure of the lung causes short-term sloughing of the epithelium and longer term fibrotic responses. Inflammatory responses are also involved. Currently, few useful therapeutic agents are available to ameliorate the adverse reactions to this agent. In vitro models of human tissues could provide faster and more cost-effective screening prior to definitive testing in an animal model. As preliminary model development, we exposed human full thickness models of the skin and airway epithelium (EpiDerm and EpiAirway cultures; MatTek Corp.) to SM vapor or to air. Half of the cultures were then transferred to the appropriate medium and the others were transferred to medium containing approximately 10 million freshly isolated human peripheral blood leukocytes. The cultures were returned to the incubator. After 24 h, the medium was harvested for analysis of matrix metalloproteinase (MMP) activity and cytokines. The cell layers were cut into two parts: one was fixed for histopathology and the other was frozen in lysis buffer. Epithelial sloughing (airway) and microblister formation (dermal) was observed in the SM-exposed cultures. The results showed upregulation of several key cytokines, with some modulation of the responses in cultures with leukocytes during the post-exposure incubations. In many cases, the responses closely mirrored responses in the lungs of rats exposed to SM vapors. MMP activity was increased in the medium as well as the cell lysates of the dermal model. These results provide preliminary information for further development of a relevant model for testing potential therapeutics.

This work was supported by Cooperative Agreement from the National Institute of Neurological Diseases and Stroke, National Institutes of Health [grant number 5U54NS058185-03].

PS 2020 PREGABALIN REDUCES THE DURATION OF ACUTE SEIZURES INDUCED BY SOMAN EXPOSURE.

M. Furtado[1], F. Rossetti[1], L. Lumley[2], M. Addis[1], M. Moffett[2], B. Robertson[1], K. Bailey[1], S. Chanda[1], N. Kelley[2], M. Stone[2], T. Pak[1], S. Lichtenstein[1] and D. Yourick[1]. *[1]Walter Reed Army Institute of Research, Silver Spring, MD and [2]U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Edgewood, MD.*

Organophosporous (OP) compounds are acetylcholinesterase inhibitors that constitute a hazard and exposure induces status epilepticus (SE), brain injury and spontaneous recurrent seizures (SRS). Long-term pregabalin treatment delays the occurrence of SRS in the lithium-pilocarpine model, protecting piriform and entorhinal cortex. Pregabalin is also used for the treatment of partial-onset seizures in patients that are nonresponsive to conventional treatments. In this study we tested if pregabalin, in combination with diazepam is effective against OP-induced seizures. Male Sprague-Dawley rats had EEG recorded through telemetry (Data Sciences International) 24 hours/day. Animals were pretreated with HI-6 (125 mg/kg, i.p.) 30 min prior to exposure to soman (110 μg/kg, s.c.) at USAMRICD. Rats were injected with atropine (2 mg/kg, i.m.) one min after exposure and diazepam (10 mg/kg, s.c.) was injected 30 min post SE onset in conjunction with pregabalin (200, 100 and 50 mg/kg i.p.) or vehicle. Animals were euthanized 15 days after exposure and brains were processed for nissl, silver and fluorojade B staining. Electrographic seizures were estimated through the use of a customized MATLAB toolbox (Mathworks, Vers. 7) and confirmed by visual screening. The preliminary findings indicated that animals treated with 200, 100 or 50 mg/kg of pregabalin had termination of initial SE earlier than the control rats (One Way ANOVA, $p<0.05$). However, both pregabalin- and vehicle-treated rats had recurrent seizures and SRS at the current tested doses and treatment schedule. We conclude that there is a need to treat not only the acute seizures induced by soman, but also the subsequent recurrent seizures in order to avoid or reduce the SRS and neuronal damage. Future long term studies are planned to further assess the efficacy of pregabalin against recurrent seizures and SRS and to quantify the number of degenerating neurons.

PS 2021 CLOT & CLEAN: *IN VIVO* DEMONSTRATION OF A NOVEL DECONTAMINATION ROLE FOR A HAEMOSTATIC DRESSING.

H. Lydon[1, 2], C. A. Hall[1, 2], J. K. Chipman[2], R. P. Chilcott[1] and J. S. Graham[3]. *[1]CBRN & Chemical Toxicology Group, Health Protection Agency, Salisbury, United Kingdom, [2]School of Biosciences, University of Birmingham, Birmingham, United Kingdom and [3]Medical Toxicology Branch, USAMRICD, Aberdeen Proving Ground, MD.*

Haemorrhage is a leading cause of death following trauma in both civilian and military contexts. Rapid response to haemorrhage is an important factor influencing survival and a number of haemostats are available commercially. In certain scenarios, treatment of haemorrhaging wounds may be complicated by the ingress of toxic materials present in the environment: for example, following the deliberate release of chemical warfare agents. Under such circumstances, a haemostatic product capable of functioning as a wound decontaminant may be of significant clinical benefit. The purpose of this study was to assess the in vivo efficacy of a granular haemostat (WoundStat™) when applied to a superficial skin wound contaminated with the organophosphate acetyl cholinesterase (AChE) inhibitor VX (S-[2-(diisopropylamino)ethyl]-O-ethyl methylphosphonothioate). Previous experiments identified that WoundStat™ retained good haemostatic efficacy in the presence of VX when assessed using pig blood in vitro; and was similar in performance to other currently in-service military decontaminants when applied to normal or damaged pig skin, evaluated using an in vitro diffusion cell system. Anaesthetised pigs (n=6) were exposed to a 5x LD50 liquid droplet of VX via a small area of damaged skin on the dorsal aspect of the pinna 30 seconds prior to application of a 2g bolus of WoundStat™ to the exposure site. Control animals (n=6) were exposed to VX but not subsequently treated. All control animals exhibited rapid onset, severe signs of poisoning and 5 animals did not survive the 6 hour exposure. Treatment with WoundStat™ reduced the severity of (or eliminated in two cases) signs of intoxication, reduced AChE inhibition, and prevented death. These results provide clear evidence that haemostatic products (based on an absorptive mechanism of action) can achieve clinically-relevant decontamination of chemical warfare agents from wounded tissue.

PS 2022 TESTING THE ABILITY OF WOUNDSTAT™ TO PROTECT AGAINST THE CHEMICAL WARFARE AGENT VX.

E. D. Clarkson[1], K. H. Smith[1], C. A. Hall[2], H. L. Lydon[2], C. H. Dalton[2], J. S. Graham[1], R. F. Railer[1], R. S. Stevenson[1], R. R. Deckert[1], J. L. Devorak[1], J. D. Boecker[1], E. H. Braue[1], H. L. Lumpkin[1], B. F. Doxzon[1] and R. P. Chilcott[2]. *[1]Analytical Toxicology Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD and [2]CBRN and Chemical Toxicology Group, Health Protection Agency, Porton Down, Salisbury, SP4 0JG, United Kingdom.*

WoundStat™ is a hemostatic agent that has been used by the US to prevent soldiers from bleeding to death in the field. This study addresses the effect that WoundStat™ would have on a bleeding wound contaminated with the highly toxic organophosphate chemical warfare agent VX. In vitro studies were conducted using pig skin placed in a diffusion cell and exposed to 14C–radiolabeled VX. Woundstat™ was one of several hemostatic agents and decontaminating agents that were tested in this in vitro model. Woundstat™ was very effective at blocking the absorption of VX over a 24-hour period of time in either intact or damaged skin. We expanded this work in vivo by first determining that the median lethal dose (MLD) of VX when placed on the ear of an anesthetized pig was 60 μg/kg. Next, 300 μg/kg of VX was applied to the ear with a glass chamber surrounding the application site and either left untreated as a control or treated with WoundStat™ with all the treated animals surviving. We then created a non-bleeding wound in the axillary area of a anethestized pig, applied neat VX into the wound, closed the wound with staples and determined the 6-hour MLD to be 28.6 μg/kg. We expanded on this work by applying 5xMLD (143 μg/kg) of VX into the wound, cutting the vein/artery bundle (which was a non-fatal bleeding wound) and when the pocket was half filled with blood pouring WoundStat™ into the pocket. Of the animals tested in this manner 3 out of 4 died prior to the 6- hour cutoff but lived longer than control animals. Thus, currently WoundStat™ fails to significantly protect against a 5xMLD challenge of VX in a bleeding wound over 6 hours but did offer a modest increase in time after exposure until death.

PS 2023 REDUCTION OF MECHLORETHAMINE TOXICITY BY STRUCTURALLY DIVERSE ANTIOXIDANTS.

A. Lulla[1], M. Pino[1], C. Lau-Cam[1], M. Pietka-Ottlik[2], J. Mlochowski[2] and B. Billack[1]. *[1]Pharmaceutical Sciences, St. John's University, Jamaica, NY and [2]Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland.*

The vesicant toxicity of the nitrogen mustard mechlorethamine (HN2) is related, in part, to an ability to deplete intracellular antioxidants since it is reduced by exogenously administered antioxidants. In the present work, several antioxidants with differing structures were investigated for the ability to reduce the toxicity of HN2 using A-431 cells, a human epidermoid carcinoma cell line. The antioxidants evaluated were butylated hydroxyanisole (BHA), resveratrol (RES), pterostilbene (PTS), vitamin C (VIT C), ebselen (EB), and a sulfur analog of EB (EB-S). In the absence of an antioxidant, a reproducible and robust cell death was observed by the MTT assay following a 24 h incubation with HN2 (25 μM), while cells treated only with an antioxidant (15, 30 or 60 μM) were generally unaffected. When the incubation was carried out in the presence of both vesicant and test antioxidant, EB was the only test compound that could rescue the cells from death as inferred from the results of light microscopic analysis and the MTT assay. However, protection by EB was only achieved when this compound was added prior to (or in co-incubation with), but not after, HN2 treatment. To determine the extent to which HN2 induces cellular death through apoptosis, cells treated with HN2 (25 μM; 8 h) were examined for their caspase-3 (CASP-3) activity. The CASP-3 activity was found to reach a maximum after 6 h and to remain elevated for at least 8 h. While a co-incubation with graded (15-60 μM) concentrations of EB led to a significant and concentration-dependent reduction in HN2-induced CASP-3 activity, the extent to which the antioxidant activity of EB might have contributed to this effect remains to be determined. Taken together, the data presented here have identified EB as a promising vesicant countermeasure *in vitro* worthy of a future investigation *in vivo*.

PS 2024 PROTECTIVE EFFICACY OF CATALYTIC BIOSCAVENGER, PARAOXONASE 1 AGAINST SARIN AND SOMAN EXPOSURE IN GUINEA PIGS.

M. Valiyaveettil, Y. Alamneh, L. Biggemann, I. Soojhawon, B. P. Doctor and M. P. Nambiar. *Closed Head Injury, Walter Reed Army Institute of Research, Silver Spring, MD.*

Human paraoxonase 1 (PON1) has been described as a catalytic bioscavenger which can hydrolyze a large amount of chemical warfare nerve agents (CWNAs) compared to the stoichiometric bioscavengers such as butyrylcholinesterase. The

goal of this study is to purify and evaluate the protective efficacy of human and rabbit serum PON1 against sarin and soman. Large amount of human and rabbit serum PON1 was purified using multiple chromatographies and characterized by gel electrophoresis and Western blotting. Purified PON1 was highly pure and exhibited catalytic activity against multiple organophosphate substrates including CWNAs. The protective efficacy of catalytically active purified PON1 was evaluated against CWNAs using a microinstillation inhalation exposure model in guinea pigs. Pretreatment with 5 - 10 units of purified PON1 showed marginal increase in the blood level but significantly increased the survival rate and reduced symptoms of soman and sarin (1.2 LCt50) exposure. The blood levels of PON1 in pre-treated animals after exposure to nerve agent was higher than that of control animals. The protective efficacy highly correlated with the increased activity of AChE and BChE in the blood and tissues of survived animals. In summary, purified human and rabbit serum PON1 efficiently hydrolyze organophosphates and CWNAs in vitro and significantly protect against sarin and soman exposure in guinea pigs. These data support the development of PON1 as an efficient catalytic bioscavenger to protect soldiers and civilians against exposure to lethal doses of CWNAs.

PS 2025 EVALUATION OF NOVEL NERVE AGENT ANTICONVULSANTS AND THEIR EFFECTS ON BRAIN GLUTAMATE CONCENTRATION IN RATS.

J. Guarisco, R. K. Kan, J. K. Chandler, A. J. Wegener, T. M. Shih and J. H. McDonough. *USAMRICD, Aberdeen Proving Ground, MD.*

High brain glutamate concentrations are observed after nerve agent poisoning and are believed to be the primary cause of nerve agent-induced neuropathology. This study was performed to determine the effects of delayed nerve agent treatment and to evaluate standard treatments plus novel anticonvulsant adjuncts on brain glutamate concentration. Rats were surgically prepared with electroencephalographic (EEG) recording electrodes and a biosensor guide cannulae directed at the basolateral amygdala. A week later the animals were pretreated with HI-6 (125 mg/kg, IP) and 30 min later challenged with the nerve agent soman (180 μg/kg, SC). EEG and a glutamate biosensor were monitored for seizure onset and brain glutamate concentration, respectively. At 20 min after seizure onset the animals received standard medical therapies, 0.45 mg/kg atropine sulfate, 25 mg/kg 2-PAM and 2.0 mg/kg diazepam, IM, either alone or with an adjunctive drug, procyclidine, ketamine or midazolam. In the absence of an adjunct drug, all animals continued to display EEG seizure activity following standard medical therapies: 8 of 8 (100%) were still seizing at 24 hr. In these animals, glutamate concentrations began to increase immediately following seizure onset, rose to levels 183% above preseizure baselines, and remained elevated for the remainder of the recording time. Adjunctive treatment with procyclidine, ketamine, or midazolam in addition to standard medical therapies stopped seizure activity even after a 20-min delay. Successful seizure control with the adjunct drugs resulted in a decrease of glutamate concentrations back to and below baseline levels over the remainder of the recording time. These data provide evidence that successful seizure control will counteract the neurotoxic increase of glutamate in the brain of soman-exposed animals.

PS 2026 THE HAIRLESS MOUSE BACK MODEL AND THE MEVM HAVE SIMILAR MOLECULAR PROFILES.

J. D. Wang[2], M. Soriano[1], N. Singer[1], R. P. Casillas[3], D. R. Gerecke[1, 2] and Y. Chang[1]. [1]*Pharmacology & Toxicology, Ernest Mario School of Pharmacy, Rutgers University, EOHSI, Piscataway, NJ,* [2]*Pharmacology & Toxicology, Ernest Mario School of Pharmacy, Joint Graduate Program in Toxicology, Rutgers University, EOHSI, Piscataway, NJ and* [3]*Battelle Biomedical Research Center, Columbus, OH.*

Sulfur mustard (bis-2-chloroethyl sulfide, SM) and Nitrogen mustard (bis-2-chloroethyl ethylamine, NM) are potent vesicant blistering agents. Both chemicals are highly reactive bifunctional alkylating agents, which crosslink proteins, DNA and other cellular components, resulting in disruption of tissue structure and loss of biological activity. We compared sulfur mustard (SM) in the Mouse Ear Vesicant Model (MEVM) to nitrogen mustard (NM) in the hairless mouse back model to study chemical irritant-induced damage over time after exposure. Our endpoints were erythema, edema, histopathology, as well as biochemical and inflammatory mediators. Both models produced statistically significant dose- and time-dependent changes in edema and histopathological features that included severe inflammation and separation of the dermis from the epidermis. Both models showed prolonged neutrophil presence and increased numbers of macrophages. Matrix metalloproteinase 9 (MMP-9) was significantly increased in both models as determined by QT-PCR and western blot analysis. Both models had significant hyperplasia at 3 days post exposure and increased expression of the keratinocyte proliferation marker, keratin 5. Expression of the wound healing protein, laminin γ2 was also similar in the two models. We conclude that NM exposure in the hairless mouse back model is a viable alternative to the MEVM exposed to SM. This work is supported by ES007148, ES005022 and AR055073.

PS 2027 USE OF GAMMA-H2AX AS A BIOMARKER TO EVALUATE THE ANTI-GENOTOXIC ACTIVITY OF COMPOUNDS IN CULTURED HUMAN SKIN CELLS FOLLOWING CEES EXPOSURE.

A. L. Miller, N. K. Waraich, C. L. Gross, E. W. Nealley, O. E. Clark, K. L. Rodgers and W. J. Smith. *Research, USAMRICD, Aberdeen Proving Ground, MD.*

CEES (2-chloroethyl ethyl sulfide) is monofunctional analog of sulfur mustard (2-2'-dichlorodiethyl sulfide, SM) a chemical warfare agent. CEES was used in this study to form a base line of DNA damage. The skin serves as a principal target site for in vivo toxicity of SM exposure resulting in the formation of blisters and inflammation. Previous studies show the genotoxic effects of SM using normal human epidermal keratinocytes (NHEK, Lonza Corp., MD) as an in vitro model by observing the presence of γ-H2AX and micronuclei formation. γ-H2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double stranded DNA break sites. Cells were exposed to 0, 50, 200, 500, 1000 or 1500 μM concentration of CEES for 2 hrs. Various compounds (anti-oxidants, anti-inflammatories, plant extracts), which were described in the literature as providing protection against the genotoxic manifestations of chemical toxicants, were added 2 hrs after CEES exposure and analyzed by flow cytometry after 24 hrs. CEES exposure resulted in a dose dependent formation of γ-H2AX. Preliminary data show that some of these compounds (N-acetylcysteine and Sulforaphane) have potential to minimize the DNA damage caused by CEES. The changes of this biomarker will be a useful technique for the study of SM toxicity and for the development of potential therapeutic approaches. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

PS 2028 ROLE OF ENDOGENOUS CANNABINOIDS IN VESICANT-INDUCED SKIN INJURY.

I. M. Wohlman[1], D. E. Heck[2], N. D. Heindel[3], M. Huang[4], D. R. Gerecke[1], P. J. Sinko[5], C. J. Lacey[3], D. L. Laskin[1] and J. D. Laskin[6]. [1]*Pharmacology & Toxicology, Rutgers University, Piscataway, NJ,* [2]*Environmental Health Sciences, New York Medical College, Valhalla, NY,* [3]*Chemistry, Lehigh University, Bethlehem, PA,* [4]*Chemical Biology, Rutgers University, Piscataway, NJ,* [5]*Pharmaceutics, Rutgers University, Piscataway, NJ and* [6]*Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

As a potent skin vesicant, sulfur mustard rapidly induces inflammation and blistering. Recent studies indicate that endogenous cannabinoids including the fatty acid amides palmitoylethanolamide and anandamide mediate dermal inflammatory reactions, a process that is down-regulated by enzymes that degrade these compounds, in particular, fatty acid amide hydrolase (FAAH). In the present studies we determined if FAAH inhibitors were effective in reducing inflammation using a mouse ear vesicant model (MEVM) and the sulfur mustard analog 2-chloroethyl ethyl sulfide. Carbamates with a fatty (aliphatic) side chain are sufficiently similar in structure to fatty acid amides [R-CO-NH-R' where R' is a lipophilic construct] that they inhibit FAAH. Two types of carbamates were evaluated as inhibitors in the MEVM, Type 1 or vanilloid-CH_2NHCOO-R and Type 2 or vanilloid-CH_2-O-CO-NHR. When R = phenyl in the Type 1 class, the IC50 for FAAH inhibition was 2.5 μM, this derivative was found to inhibit inflammation by more than 75% in the MEVM. Conversely, when R = methoxyethyl there was no activity against FAAH and minimal effects on inflammation in the MEVM. When R = geranyl in the Type 2 class, the IC50 was 140 μM, inflammation was suppressed 34%; when R = phenoxyformyl, the IC50 was 120 μM and the inflammation suppression was 62%; when R = perillyl, the IC50 was 17 μM and inflammation suppression 53%. These results indicate that FAAH inhibitors are active as suppressors of vesicant-induced inflammation in the MEVM and may be useful as sulfur mustard countermeasures. Moreover, endocannabinoids appear to be important mediators of vesicant-induced inflammatory reactions in the skin. Supported by AR055073, ES004738 and ES005022.

PS 2029 EFFICACY OF ENDOTRACHEAL ADMINISTRATION OF ADENOSINE RECEPTOR A1 ANTISENSE OLIGONUCLEOTIDE (EPI 2010) IN COMBINATION WITH ATROPINE METHYL BROMIDE AGAINST RESPIRATORY TOXICITY FOLLOWING MICROINSTILLATION INHALATION EXPOSURE TO SARIN.

J. Song[1], P. Rezk[2], M. Perkins[2], P. Sabnekar[2], S. Oguntayo[1], A. M. Scuito[2], B. P. Doctor[1] and M. P. Nambiar[1, 3]. *[1]Closed Head Injury Branch, Center For Military Psychiatry and Neuroscience, Walter Reed Army Institute of Research, Silver Spring, MD, [2]Medical/Analytical Toxicology, U.S. AMRICD, Aberdeen Proving Ground, MD and [3]Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD.*

To develop nasal therapeutics for nerve agnet exposure, we evaluated the efficacy of endotracheal administration of a respirable antisense oligonucleotide that blocks the synthesis of the adenosine A1 receptor (EPI 2010) against sarin inhalation exposure. EPI 2010 attenuates bronchoconstriction, inflammation and surfactant depletion. EPI 2010 was combined with atropine methyl bromide (AMB), an antimuscarinic agent that blocks airways secretion which is critical for protection. Age and weight matched male guinea pigs treated with 60 mg/kg HI-6 oxime were exposed to 846.5 mg/m3 sarin using microinstillation inhalation exposure technique for 4 min. Twenty minutes later, the animals were treated with endotracheal EPI 2010 (10 pmol) combined with AMB (2.5 mg/kg) or saline control. Treatment with the combined drug aerosol significantly decreased symptoms of agent exposure and increased the survival from 73% (untreated sarin controls) to 100%. Decreased blood O2 saturation and heart rate following sarin exposure were also significantly improved by the combination treatment. Inhibition of blood AChE and BChE activities following sarin exposure was also significantly reduced in animals with the treatment. Increase in total bronchoalveolar lavage fluid protein after sarin exposure was also reduced in the combination treated groups. Respiratory dynamics including minute volume, peak inspiration flow, time of inspiration, time of expiration and end of aspiratory pause at 4 h after exposure were returned to normal level after the treatment. These data suggest that endotracheal administration of EPI 2010 along with AMB protects against sarin exposure and increase survival.

PS 2030 CELL SURVIVAL AND APOPTOSIS MARKERS IN SULFUR MUSTARD EXPOSED MOUSE SKIN.

Y. Chang[1], J. D. Wang[1], R. P. Casillas[2], M. K. Gordon[1] and D. R. Gerecke[1]. *[1]Department of Pharm and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Pisacataway, NJ and [2]Battelle Biomedical Research Center, Columbus, OH.*

Endoplasmic reticulum stress response (ERS) is a cell survival mechanism that occurs whenever a cell is under severe stress. A severely damaged cell such as an epithelial cell exposed to sulfur mustard (SM) upregulates specific chaperone proteins that target misfolded and incorrectly made proteins to the proper compartments for degradation. In this way the injured cell may be targeted for the cell survival pathway rather than apoptosis. Time course studies of the vesicant agent, SM, in the mouse ear vesicant model (MEVM) showed histopathology changes over time and an induction of the ERS response as determined by the upregulation of specific genes. Changes in SM exposed skin included edema, separation of the epidermis from the dermis, prolonged inflammation and delayed wound healing as time progressed from 24h to 168h. RT-PCR and western blot analysis of the ER stress genes, GRP 78 and CHOP demonstrated at least 2 fold increased expression as time progressed. This was verified by immunofluorescence of paraffin tissue sections. An increase in the Endoplasmic reticulum (ER) stress mediated non-mitochondrial apoptosis pathway also occurred as determined by activation of caspase-12 and the downstream product, cleaved-caspase 3, over time. Double immunofluorescence labeling of the keratinocyte migration promoting protein, laminin γ2, and GRP78, showed colocalization of the two molecules at 72h post exposure suggesting that the laminin γ2 was misfolded after SM exposed skin and trapped in the ER rather than secreted as expected. Taken together, our observations demonstrate that both ERS and apoptosis occur in the skin after SM exposure. This work is supported by ES005022, EY09056, and AR055073.

PS 2031 SULFUR MUSTARD EXPOSURE OF THE CORNEA INDUCES AUTOPHAGY.

L. Raman[1], R. A. Hahn[1], J. J. Schlager[2], R. Gordon[3], D. R. Gerecke[1], M. C. Babin[4] and M. K. Gordon[1]. *[1]Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, [2]Air Force Research Laboratory, Wright-Patterson AFB, Dayton, OH, [3]Pathology, Mount Sinai School of Medicine, New York, NY and [4]Battelle Biomedical Research Center, West Jefferson, OH.*

Sulfur mustard (SM) injury to the cornea can lead to blindness. SM is an alkylating agent known to crosslink proteins in the epidermal basement membrane extracellular matrix (Zhang Z, Peters BP, Monteiro-Riviere NA. Assessment of sulfur mustard interaction with basement membrane components. Cell Biol Tox. 11:89-101, 1995). This is expected to be true also for the cornea. Therefore, whether autophagy might be a mechanism for degrading corneal SM-alkylated proteins was examined. Neat sulfur mustard (0.4 microliter) was applied to the central corneas of New Zealand white rabbits. Rabbits were sacrificed at 1 and 3 days post-exposure, and the corneas were dissected, embedded in epon, and sectioned for analysis by transmission electron microscopy (TEM). Unexposed rabbit eyes were used as controls. TEM analysis of a minimum of 100 epithelial and 100 stromal cells at each time point revealed that both unexposed and SM-exposed corneal cells contained autophagosomes, and that these structures were more prevalent in the SM-exposed cells. One day after SM exposure 100 corneal epithelial cells have 52% more autophagosomes than 100 unexposed corneal epithelial cells, however the number in the exposed stromal cells was the same as in unexposed corneas. At 3 days post SM exposure, 100 exposed epithelial cells have 616% more autophagosomes than unexposed epithelia, and 100 stromal cells have 424% more than 100 unexposed corneal stromal cells. The results of this semi-quantitation suggest that autophagy in SM-exposed corneas is likely one mechanism used by cells to degrade vesicant-alkylated proteins. Supported by RO1 EY009056, U54 AR055073 and P30 ES005022.

PS 2032 NITROGEN MUSTARD-INDUCED CORNEAL INJURY IS AMELIORATED BY ADAM17/TACE INHIBITORS.

A. S. DeSantis[1], R. A. Hahn[1], N. D. Heindel[2], K. K. Svoboda[3], D. R. Gerecke[1] and M. K. Gordon[1]. *[1]Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, [2]Chemistry, Lehigh University, Bethlehem, PA and [3]Biomedical Sciences, Baylor College of Dentistry, Texas A&M Health Science Center, Dallas, TX.*

Nitrogen mustard (NM) causes epithelial-stromal separation in the cornea. We found that NM exposure increases ADAM17 (aka TNFα converting enzyme, TACE) activity, which results in cleavage of transmembranous collagen XVII, a component of the hemidesmosome and anchoring complex. To determine whether rabbit corneal organ cultures treated with NM would heal faster if ADAM17/TACE inhibitors were applied, rabbit corneas were placed in air lifted organ cultures. Medium was added up to the corneal-scleral junction. 100 nmoles of NM were applied onto central corneas. After 2 hr, contaminated medium was replaced with fresh medium alone, or with medium plus one of four ADAM17/TACE inhibitors. The inhibitors were applied a total of 4 times over the course of 22 hours. Corneas were analyzed by light and immunofluorescence microscopy, western blotting, and by ADAM17 activity assays. Sections of unexposed corneas showed a continuous line of immunofluorescent signal at the basement membrane zone (BMZ) with a collagen XVII monospecific antibody, which disappeared 24 hr after vesicant exposure, indicating cleavage of collagen XVII. H&E staining of NM-exposed corneas confirmed epithelial-stromal separation. 22 hr of treatment with an ADAM17/TACE inhibitor, starting at 2 hr post exposure, showed fewer separations, and a collagen XVII antibody pattern similar to unexposed corneas. An ADAM17 extracellular domain antibody reacted strongly after NM exposure, but the signal was greatly attenuated in sections treated with an inhibitor. In conclusion, vesicant exposure leads to activation of ADAM17/TACE, which cleaves collagen XVII at the epithelial-stromal junction. The 2 hr post-exposure application of ADAM17/TACE inhibitors attenuates loss of the epithelium from the stroma and preserves the integrity of the BMZ. Supported by RO1 EY009056, U54 AR055073 and P30 ES005022.

PS 2033 COMPARISON OF CORNEAL WOUND HEALING AFTER ULTRAVIOLET AND NITROGEN MUSTARD INJURY.

I. P. Po[1], A. S. DeSantis[1], R. A. Hahn[1], D. R. Gerecke[1], J. D. Laskin[2] and M. K. Gordon[1]. *[1]Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ and [2]Environmental and Occupational Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Vesicants such as nitrogen mustard (NM) cause blistering of skin, similar to that caused by ultraviolet B (UVB) exposure from the sun. In the cornea, such injury occurs as microbullae of the basal epithelial cells bordering the basement mem-

brane. Our goal is to determine whether a nitrogen mustard injury heals more slowly than an equivalent UVB injury. To accomplish this, corneal organ cultures were exposed to different levels of UVB to determine conditions that would produce a 24 hr post-UVB phenotype equivalent to the 24 hr post-exposure phenotype of a 60 min exposure to 100 nmol NM. Corneal organ cultures were irradiated 14 cm from the light source for various times to produce different intensities of UVB: 5 min resulted in 100 mJ/cm2, 20 min, 400 mJ/cm2; 40 min, 800 mJ/cm2; 60 min, 1200 mJ/cm2; 80 min, 1600 mJ/cm2; and 100 min resulted in a 2000 mJ/cm2 exposure. To compare these organ cultures to those exposed for 60 min to 100 nmol NM, corneas were embedded in O.C.T. medium, frozen, then sectioned for H&E staining. Micrographs of sections were overlapped to make composites covering the entire diameter of the cornea. From the composites, the length of denuded cornea was calculated as a percentage of the entire width. Our results demonstrated that a 60 min exposure to 100 nmol NM resulted in the epithelium separating from the stroma across 58.3% of the corneal diameter. A nearly equivalent phenotype (producing a 61.7% separation between the cell layers) resulted from the 2000 mJ/cm2 UVB exposure. The time for the recovering epithelium to completely migrate over the stroma was determined, and was shown to take 4 days after NM exposure. In contrast, the equivalent damage from UVB exposure recovered in only 3 days. Supported by RO1 EY009056, U54 AR055073 and P30 ES005022.

PS 2034 SIGNALING MECHANISMS THAT CAN CONTRIBUTE TO THE NEUROPROTECTION AFFORDED BY GALANTAMINE IN GUINEA PIGS EXPOSED TO THE NERVE AGENT SOMAN.

G. Kulkarni[1], M. Akkerman[1], Y. Aracava[1], E. X. Albuquerque[2, 1] and E. R. Pereira[1, 2]. [1]*Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD and* [2]*Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD.*

Treatment of guinea pigs with galantamine, a drug used to treat Alzheimer's disease, prevents the neurodegeneration induced by organophosphorus nerve agents, including soman [PNAS 103:13220, 2006]. This study was designed to identify mechanisms that may contribute to the effectiveness of galantamine. Male guinea pigs (30-32 days old) were treated with: (i) saline (0.5 ml/kg, im), (ii) galantamine (8 mg/kg, im), (iii) soman (28 μg/kg, sc), (iv) galantamine followed 30 min by soman, or (v) soman followed 15 min by galantamine. All animals, except those challenged with soman alone, survived. Only 50% of the soman-challenged, untreated guinea pigs survived; about 75% of them had mild or no signs of intoxication whereas the remaining 25% presented severe signs of acute toxicity. At various times after each treatment, animals were euthanized, and their brains removed for subsequent protein analysis. At 2 h after treatment with galantamine, levels of heat shock protein 25 (Hsp-25), and ratios of phospho-Akt to total Akt and phospho (ser-136) Bad to total Bad were higher than those measured in brain extracts from saline-injected animals. Both Hsp-25 and phospho (ser-136) Bad are known to promote cell survival. Levels of Hsp-25 were also found to be elevated in brain extracts obtained at 2 h after the soman challenge of guinea pigs that survived with only mild or no signs of acute toxicity. This may represent a compensatory mechanism triggered by the stress induced by soman. Brain levels of Bcl-2 progressively decreased from 2 to 24 h after the soman challenge. Treatment of the guinea pigs with galantamine prevented soman-induced loss of Bcl-2. The results presented herein provide a mechanistic basis for the neuroprotection afforded by galantamine in animals exposed to soman. (Support: NIH Grant UO1NS059344)

PS 2035 GALANTAMINE COUNTERACTS COGNITIVE DEFICITS IN GUINEA PIGS CHALLENGED WITH THE NERVE AGENT SARIN.

J. Mamczarz[1], Y. Aracava[1], E. R. Pereira[1, 2] and E. X. Albuquerque[2, 1]. [1]*Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD and* [2]*Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD.*

Epidemiological studies have reported chronic memory decline among victims and first responders of the 1995 Tokyo terrorist attack with the nerve agent sarin (Environ Health Perspect 109:A542, 2001; Environ Health Perspect 109:1169, 2001). This study was designed to test the hypothesis that a single exposure of guinea pigs to a low dose (0.6xLD50) of sarin induces delayed cognitive impairments that are counteracted by treatment with galantamine, a drug that protects guinea pigs against the acute toxicity of nerve agents (PNAS 103:13220, 2006). Prepubertal male guinea pigs received: (i) saline (0.5 ml/kg, im), (ii) sarin (25.5 μg/kg, sc), or (iii) sarin followed 1 h later by galantamine (8 mg/kg, im). Three months after the injections, the cognitive performance of the guinea pigs was first tested in the Morris water maze (MWM). Animal received training to a fixed platform position. Training consisted of four consecutive trials a day over 6 consecutive days followed by the probe test on day 7. Compared to saline-injected animals, sarin-challenged guinea pigs showed slower acquisition of the MWM task; only on the 6th day of training did sarin-challenged animals perform as well as control animals. There was also no significant difference in the probe test between sarin- and saline-injected guinea pigs. In the 8-arm radial water maze, the performance of sarin- and saline-injected animals was comparable. Thus, the cognitive impairment developed by sarin-challenged animals is related to the procedural component of learning. Sarin-challenged guinea pigs that were treated with galantamine presented no cognitive impairment in any test. It is, therefore, concluded that: (i) a single exposure to a low dose of sarin triggers the development of cognitive deficits, and (ii) galantamine is an effective antidote to counteract the delayed neurotoxic effects of sarin. (Support: NIH Grant UO1NS059344)

PS 2036 ANALYSIS OF MAP KINASE SIGNALING IN SULFUR MUSTARD TOXICITY USING PHARMACOLOGIC INHIBITORS AND GENE EXPRESSION PROFILING.

C. C. Rothwell, C. M. Crum, P. A. Everley and J. F. Dillman. *Cell and Molecular Biology, USAMRICD, Aberdeen Proving Ground, MD.*

Sulfur mustard (SM) is a potent alkylating agent and vesicant. SM exposure activates numerous signaling cascades such as the mitogen-activated protein kinase (MAPK) cascades. These include the ERK, p38, and JNK pathways, which are involved in cell growth, inflammation, and stress signaling. The precise roles of these pathways in SM toxicity have not been elucidated. We used Western blotting along with pharmacological inhibitors and microarray analysis to examine the activation and role of each pathway after SM exposure in primary human keratinocytes. Western blotting revealed increased phosphorylation of p38 and JNK after SM exposure; however phosphorylation of ERK was equivocal, suggesting that growth conditions may impact ERK activation by SM. We used pharmacologic inhibitors to target each MAPK and then compared gene expression profiles to identify SM-induced genes regulated by each MAPK. Cells were pretreated with SB203580 (p38 inhibitor), PD98059 (ERK inhibitor), or SP600125 (JNK inhibitor) before SM exposure. Cells were harvested at 1, 4, and 8h post-exposure, and RNA was extracted for gene expression profiling. Analysis of variance identified genes significantly modulated due to pharmacologic inhibition in SM-exposed cells. Pathway mapping confirmed alterations in SM-induced MAPK signaling due to pharmacologic inhibition. SM-induced expression of IL-6, IL-8, and TNF-alpha inflammatory cytokines was decreased by p38 MAPK inhibition, but not by inhibition of other MAPKs. Based on the number of significant pathways mapped to each MAPK in the presence and absence of inhibitors, the p38 pathway appeared to be the MAPK cascade most responsive to SM. Interestingly, pathway mapping of the microarray data identified potential cross-talk between MAPK pathways and other pathways involved in SM-induced signaling. Mining of these results will increase our understanding of the role of MAPK cascades in SM-induced signaling and may identify additional therapeutic targets for the development of SM medical countermeasures.

PS 2037 THE NEUROPROTECTANT EFFECTS OF DIAZEPAM ALONE AND COMBINATION WITH HISTONES DEACETILASES INHIBITORS IN A RAT MODEL OF STATUS EPILEPTICUS INDUCED BY ORGANOPHOSPHORUS EXPOSURE.

F. Rossetti[1], M. Furtado[1], M. Addis[1], B. Robertson[1], M. Moffet[2], L. Lumley[2] and D. Yourick[1]. [1]*Walter Reed Army Institute of Research, Silver Spring, MD and* [2]*U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.*

The occurrence of status epilepticus (SE) is considered the main cause of brain lesions and morphological alterations that could result in chronic epilepsy. SE caused by nerve agents such as soman (organophosphorus family of chemicals) begins rapidly, continues for hours, and contributes to prolonged physical incapacitation and neuropathology in both rodent and non-human primate species. Identification of neuroprotectants with efficacy against nerve agent-induced brain injury is the subject of the present study. Male Sprague-Dawley (n=69) had SE-induced by LD50 of soman (110 μg/kg, s.c.) and neuropathological effects analyzed 72 hours after.

Diazepam injections and its combination with the histone deacetylase (HDCA) inhibitors, suberoylanilide hydroxamic acid (SAHA) and valproic acid (VPA) were administered and analyzed. Neuropathology was assessed via FluoroJade B staining. The extent of brain damage induced by SE is dependent upon maximum seizure score attained in the period directly after soman exposure; Diazepam treatment, with or without HDAC inhibitors, was not protective of neuronal loss in piriform nuclei; The combination diazepam/SAHA (25 mg/Kg) was more neuroprotective in hippocampus than just diazepam alone; Both SAHA 50 mg/Kg and VPA 25 mg/kg injections increased neuronal loss in thalamus and cortex; Diazepam also prevented some neuronal loss in the Substantia Nigra Pars Reticulata (SNPr). Soman-induced SE caused brain structure-specific injury 72 hours after SE onset. The damage appears in all nuclei of the hippocampus, amygdala, thalamus, piriform and other cortical regions and is not restricted to limbic areas. Soman-induced brain damage is widespread and complex, is differentially affected by combinations of diazepam and HDAC inhibitors, and detailed studies are needed to identify mechanisms and potential.

PS 2038 ACUTE CL2 INHALATION CAUSES NEUTROPHIL RECRUITMENT AND SURFACTANT INACTIVATION IN MICE.

C. B. Massa, C. J. Guo, P. Scott and A. J. Gow. *Toxicology, Rutgers University, Piscataway, NJ.*

Chlorine gas has been identified as a potential threat to both civilian and military populations. Acute exposure to high levels of Cl2 gas can cause respiratory dysfunction, which often progresses to severe respiratory distress and death. Much of the available literature describes chronic, low-dose exposure to volatile chlorinated compounds other than authentic Cl2 gas; however the pathology of acute exposure is less well known. We proposed that acute Cl2 exposure leads to direct modification of the components of the lung lining fluid, resulting in surfactant dysfunction and inflammation as initiating events in acute respiratory distress. C57/BL6 mice were exposed to 300 ppm Cl2 for 1 hour. Measures of lung function, surfactant inactivation and inflammation were compared to naive controls at 3, 24 and 48 hrs post injury. Forced oscillation measurements showed increases in lung elastance and altered lung recruitment, with the 24 hr time point being most effected. Contrary to data chronic exposure, there was no evidence of airway constriction. Capillary surfactometry on the Bronchoalveolar lavage (BAL) fluid demonstrated an increase in opening pressure post Cl2, indicating reduced surface-active function. Examination of surfactant protein expression by western blot showed an increase in SP-D with a concomitant loss of SP-B. These changes may mediate the loss of surface activity in the BAL. As well as increased SP-D levels increased cell number within the BAL following exposure indicates the induction of inflammatory processes. BAL cell infiltrate demonstrated a profound neutrophillic component in addition to an increase in macrophage number. BAL protein was not increased, suggesting intact epithelial barrier function. RT-PCR on BAL cells showed increased expression of the Arg1, CCL2, FIZZ1, IL-1b, and PTGS2 genes, with peak change in all genes at 24 hrs. Neither BAL iNOS mRNA or protein were increased, nor were NO3- or NO2-. Whether a common mechanism underlies surfactant inactivation and the inflammatory response is under investigation. NIH HL-086621, ES-005022, ES-007148.

PS 2039 DURATION OF STATUS EPILEPTICUS DOES NOT AFFECT LEVEL OF BRAIN DAMAGE IN SOMAN EXPOSED RATS.

K. Bailey[1], L. Wierenga[1], L. Yang[1], L. West[1], C. Wheeling[1], M. Furtado[1], D. Yourick[1] and L. Lumley[2]. [1]*Walter Reed Army Institute of Research, Silver Spring, MD and* [2]*U.S. Army Medical Research Institute of Chemical Defense, Edgewood, MD.*

Soman is an organophosphate nerve toxin that inhibits the breakdown of the excitatory neurotransmitter acetylcholine by inhibiting acetylcholinesterase. In rats, exposure to the LD50 (100 μg/kg in 1 ml) of soman can induce status epilepticus (SE), resulting in varying levels of brain damage. Cortical electroencephalographic (EEG) readings and qualitative assessments of regional brain damage can be used to evaluate seizure activity and severity of injury as a result of soman exposure. With the aid of custom MATLAB software that identifies frequency and duration of EEG seizures, comparisons were made between seizure activity and neuropathology measured by computer-aided analysis of silver stained coronal brain sections. We find that there is no significant correlation between duration of EEG seizures during status epilepticus and qualitative measurements of the hippocampus (R2 = 0.19), thalamus (R2 = 0.26), amygdala (R2 = 0.003), piriform cortex (R2 = 0.02), or cortex (R2 = 0.21) in rats euthanized three days after exposure to soman. The lack of significant correlation between the duration of SE and severity of brain damage in rats exposed to soman suggests that antiseizure treatments are not sufficient for minimizing the brain damage suffered from an organophosphate nerve toxin insult.

PS 2040 TWO-HIT MODEL OF CEES SKIN INJURY INVOLVING DNA DAMAGE AND INDUCTION OF INFLAMMATORY MEDIATORS, IN PART *VIA* OXIDATIVE STRESS, IN SKH-1 HAIRLESS MOUSE SKIN.

A. K. Jain[1], N. Tewari-Singh[1], M. Gu[1], S. Inturi[1], C. W. White[2] and R. Agarwal[1]. [1]*Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO and* [2]*Pediatrics, National Jewish Health, Denver, CO.*

Sulfur mustard (SM), a bifunctional alkyalating agent, causes severe cutaneous injury characterized by inflammation and delayed blistering. Our recent studies have found that 2-chloroethyl sulfide (CEES), an analog of SM, causes microblisters, inflammation and histopathological alterations similar to SM in SKH-1 hairless male and female mouse skin models. We have also shown that CEES-induced oxidative stress is the major cause of skin inflammatory response, which is via the activation of Akt/MAP kinase and AP1/NF-κB pathways. To further define the mechanisms of CEES-caused skin injury and to establish a link of CEES-induced signaling pathways with its inflammatory responses, here we performed detailed molecular analysis of SKH-1 hairless mouse skin samples exposed to 2 or 4 mg CEES dose topically. Immunoblot analysis showed strong increase in the expression of COX2 (6.5 fold), iNOS (4.1 fold), MMP-9 (5.2 fold) in CEES-treated skin samples compared to controls indicating their involvement in CEES-caused inflammatory responses. A strong phosphorylation of H2A.X at ser139 (3.4 fold) was also observed showing the involvement of DNA damage in CEES-caused skin injury, which could be in part due to CEES-caused oxidative stress reported recently by us in this mouse model. To confirm the role of CEES-caused oxidative stress in the observed DNA damage and induction of inflammatory molecules, mice were given glutathione (GSH) by oral gavage 1 h before CEES exposure. GSH treatment significantly reduced increase in both CEES-caused H2A.X phosphorylation (60%) as well as expression of COX2 (70%), iNOS (52%) and MMP-9 (54%), which possibly led to the observed attenuation in CEES-caused inflammatory responses by GSH reported by us recently. Collectively, our results clearly show a two-hit model of CEES-caused skin injury involving DNA damage and induction of inflammatory mediators, at least in part via oxidative stress, in SKH-1 hairless mouse skin.

PS 2041 EFFICACY OF ENDOTRACHEAL AEROSOLIZATION OF SCOPOLAMINE FOLLOWING MICROINSTILLATION INHALATION EXPOSURE TO SOMAN IN GUINEA PIGS.

M. W. Perkins[1], Z. Pierre[1], P. Rezk[1], P. Sabnekar[1], J. Song[2], S. Oguntayo[2], A. Sciuto[1], B. Doctor[2] and M. P. Nambiar[2]. [1]*Analytical Toxicology, U.S. AMRICD, Aberdeen, MD and* [2]*Closed Head Injury Branch, Walter Reed Army Institute of Research, Silver Spring, MD.*

Soman (GD) is one of the most toxic chemical warfare nerve agents (CWNA), because it inactivates acetylcholinesterase (AChE) and the complexe 'ages' within minutes making it difficult to reactivation by oximes. There are limited studies investigating respiratory toxicity following inhalation exposure to GD, a most predicted route of exposure, if the agent is used in war or terrorism. We are studying respiratory toxicity following endotracheal microinstillation inhalation exposure to GD and evaluating post exposure therapeutics for protection. Here we report the efficacy of post-exposure treatment with aerosolized scopolamine, a muscarinic antagonist that is more effective than atropine against GD exposure. An endotracheal microinstillation inhalation exposure technique was used to aerosolize GD 841 mg/m3 (1.3 LCt50) or saline to anesthetized male guinea pigs and the animals were treated with scopolamine (0.25 mg/kg) 1 min post-exposure. Treatment with scopolamine prevented the reduction in pulse rate and blood O2 saturation and significantly increased the survival in animals exposed to GD. Increased body weight loss returned to controls level in treated animals. Increased bronchoalveolar lavage protein and cell death was returned to normal the AChE and BChE activity remained higher in scopolamine treated animals compared to controls. Post-exposure treatment with nasal scopolamine reduced the changes in various respiratory parameters including bronchoconstriction (Pause) and lung resistance (Penh), at 4 h and persisted 24 h post GD exposure. These results suggests that post-exposure treatment by endotracheal aerosolization of scopolamine protects against lethal dose GD exposure.

PS 2042 MECHANISTICALLY-BASED COMPUTATIONAL MODEL OF THE HOST IMMUNE RESPONSE TO BIOLOGICAL WARFARE AGENTS: APPLICATION TO TULAREMIA.

C. Hack, E. J. Fleming, P. E. Anderson and J. M. Gearhart. *AFRL/711 HPW/RHPB, Wright-Patterson Air Force Base, OH.*

Category A pathogens are highly virulent, top priority threat agents, yet there is no effective vaccine for several, and the best animal model remains to be determined for some. There is a clear need for tools for investigating mechanisms of pathogenesis in multiple species and exploring intervention strategies. We are developing a mechanistically-based computational model of the interactions between pathogens and the host immune system. The model structure incorporates cellular members of innate and adaptive immunity as well as cytokines to orchestrate their actions. Our computational approach allows various steps in immune response to be turned up or down, to simulate pathogen-specific mechanisms of immune subversion. In applying the model to tularemia, simulated production of pro-inflammatory cytokines by infected macrophages and dendritic cells was reduced by strain-dependent amounts. The resulting time course profiles of neutrophils, macrophages, dendritic cells, helper T cells, B cells, cytotoxic T lymphocytes, natural killer cells, and bacterial cells were compared with data from experimental mouse models of tularemia. The results show successful simulation of pathogen-host response dynamics, and suggest the present research could be used to help identify and develop novel interventions that focus on interrupting the disease cycle through incorporation of prophylactic and therapeutic agents into the model. Ultimately, this work will provide useful decision-making tools and answers to biodefense questions, particularly those that are difficult to answer using other approaches such as risk to humans and enhanced virulence of genetically altered pathogens.

PS 2043 ABSORPTION, DISPOSITION ELIMINATION KINETICS OF THE BIOLOGICAL TOXIN MYCROCYSTIN LR.

D. Kracko, K. Turteltaub, R. Harris, M. Doyle-Eisele and J. McDonald. *Lovelace, Albuqueruque, NM.*

The objective of this research is to define elimination kinetics/disposition of biomarkers of exposure to Microcystin LR. This is conducted through tissue distribution and pharmacokinetic studies in rodents and non-human primates (NHP), modeling of disposition and elimination characteristics, selection of optimal excretion matrix to utilize for biomarker targeting, and identification of biomarkers that can be used to link to toxin exposure. The rodent data are described here. Custom 14C labeled toxin was developed that allows disposition and excreta analysis. Both radioactivity and ELISA analysis of parent compound were conducted. Sprague-Dawley rats were observed up to 72 hf post oral dose administration. Tissues and blood were collected at 0.25, 1, 4, 8, 12, 24, 48, 72 hr. Urine and feces were collected for 72 h. SEB showed rapid absorption from the GI tract, and metabolism of the parent compound within 1-2 hours after dose administration. The metabolism was confirmed by the absence of parent compound coupled to the presence of radioactivity remaining in tissues, plasma and excreta. Microcystin LR showed concentrations of parent compound in excreta and peripheral organs. It was detected in both urine and feces 24-72 hr after dose administration. Interestingly, the radioactivity did show excretion at earlier time points. These results may suggest kidney malfunction, organ overload, or some other compromise of the endogenous proteolytic degradation mechanism. The intact or fragmented Microcystin was observed in the GI and peripheral organs, especially the kidney and liver. Urine samples showed high concentrations of intact Microcystin LR, and thus may be an appropriate matrix to assess for biomarkers. High levels of Microcystin LR metabolites were observed in feces samples, allowing it to be another appropriate matrix to assess for potential biomarkers.

PS 2044 ABSORPTION, DISPOSITION ELIMINATION KINETICS OF THE BIOLOGICAL TOXIN SEB.

R. Harris, M. Doyle-Eisele, J. McDonald and K. Turteltaub. *Lovelace, Albuqueruque, NM.*

The objective of this research is to define elimination kinetics/disposition of biomarkers of exposure to Staphylocollal Enterotoxin B (SEB). This is conducted through tissue distribution and pharmacokinetic studies in rodents and non-human primates (NHP), modeling of disposition and elimination characteristics, selection of optimal excretion matrix to utilize for biomarker targeting, and identification of biomarkers that can be used to link to toxin exposure. The rodent data are described here. Custom 14C labeled toxin was developed that allows disposition and excreta analysis. Both radioactivity and ELISA analysis of parent compound were conducted. Sprague-Dawley rats were observed up to 72 h post oral dose administration. Tissues and blood were collected at 0.25, 1, 4, 8, 12, 24, 48, 72 hr. Urine and feces were collected for 72 h. SEB showed rapid absorption from the GI tract, and metabolism of the parent compound within 1-2 hours after dose administration. The metabolism was confirmed by the absence of parent compound coupled to the presence of radioactivity remaining in tissues, plasma and excreta. Radioactivity from SEB metabolites spiked in plasma and urine at approximately 6-10 hours after dosing. By 72 hours post dose, urine and plasma concentrations were near zero. Tissue uptake was observed early (3 hr) in liver, followed by an increase in kidney. In all cases the absorption from the stomach was followed by a peak in concentration within the first 12 hours that was then followed by a in tissue concentration and excretion. For evaluation of biomarkers, the peak concentration would be obtained within the first 12 hrs after dosing. However, it appears that metabolites from SEB exposure may be detectable out to 72 hr post exposure. Based on these results, biomarkers of exposure focused on the measurement of intact SEB would not be feasible. However, detection of metabolites may offer a unique opportunity to assess exposure.

PS 2045 CORRELATION OF LD50 AND CYTOCHROME C OXIDASE ACTIVITY IN MITOCHRONDRIA FROM BRAINS OF RODENTS TREATED WITH CYANIDE AND CYANIDE POISONING ANTIDOTES.

D. Haines, I. Petrikovics, P. Guidry and M. Marziaz. *Department of Chemistry, Sam Houston State University, Huntsville, TX.*

The mechanism of cyanide's toxicity remains an active area of study despite many years of intense research. It has been well established that this small toxin inhibits mitochondrial electron transport by inhibiting cytochrome c oxidase, but additional and more subtle targets appear to exist that contribute to toxicity as well. We have measured cytochrome c oxidase activity in the brains of mice and rats given lethal or sub-lethal cyanide doses, either alone or in the presence of potential therapeutic and prophylactic agents. The results support earlier claims that cytochrome c oxidase is inhibited but that maximal inhibition is less than 80% of the total cytochrome c oxidase activity. Although the data for individual rodents varies significantly as is normal for animal studies, we observe excellent correlation between the EC75 for cytochrome c oxidase inhibition determined for each treatment regimen with the corresponding LD50 values determined from rodent survival for the the same regimen. These studies were supported by the ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE under the auspices of the U.S. Army Research Office Scientific Services Program and the Welch Foundation.

PS 2046 NOVEL SULFUR DONOR FORMULATIONS FOR CYANIDE ANTAGONISM.

P. K. Jayanna[1], I. Petrikovics[1], J. C. Yu[2], M. Zottola[3], M. Ancha[1] and G. A. Rockwood[3]. [1]*Chemistry, Sam Houston State University, Huntsville, TX,* [2]*Forensic Science, Sam Houston State University, Huntsville, TX and* [3]*Analitical Toxicology, U.S. AMRICD, Aberdeen Proving Ground, MD.*

The major mechanism to detoxifying cyanide in the body is through the formation of thiocyanate, which is then readily excreted in urine. Our antidotal approach is based on the utilization of exogenously administered sulfur donors and sulfurtransferases, e.g. rhodanese. Sulfur donors with better lipophilicities (better membrane penetration capabilities) can utilize the endogenous rhodanese localized within the mitochondria. Dimethyltrisulfate (DMTS) reacts effectively with cyanide to yield thiocyanate. Earlier studies reported the liposomal encapsulations of DMTS with exogenous rhodanese, and their in vivo antidotal efficacy. The present research was directed towards evaluating the hypothesis that micellar incorporation of DMTS alone would also be advantageous: namely by enhancing the membrane penetrating ability of the DMTS and by increasing the pre-administration stability by mitigating the volatility of DMTS in solution. Accordingly, we prepared and characterized PEG-PE micellar DMTS, an appropriate formulation for intramuscular administration in vivo. The micelles were characterized by cryo-electron microscopy. Due to the fact that in micelles DMTS molecules are completely immersed the hydrophobic lipid core, a high encapsulation (70-85%) efficiency was achieved. Head Space GC-MS experiments demonstrate that the micellar DMTS is significantly more stable than DMTS by itself. We propose that micellar DMTS represents a feasible sulfur donor formulation with in vivo applicability.

PS 2047 ESTERASE ACTIVITIES AND HEMOSTASIS OF RAT BLOOD AT DIFFERENT STAGES AFTER ACUTE INTOXICATION WITH WARFARE ORGANOPHOSPHATES.

N. Goncharov, V. Shmurak, V. Garnyuk, I. Kurdyukov, A. Nadeev, N. Voitenko, D. Prokofieva, Y. Pechenevskii, A. Radilov and V. Rembovskiy. *RIHOPHE, Saint Petersburg, Russian Federation.* Sponsor: R. Gupta.

Earlier observations upon victims of acute and chronic intoxications with warfare organophosphates revealed vegetative changes of cardiovascular system and "microorganic disorders" of the CNS of uncertain etiology. There were grounds to believe that vascular pathology plays a significant role in determining delayed effects of acute poisoning. The aim of present work was to investigate dynamic changes of esterase activity, some other enzyme activities and hemostasis of rat blood at different stages after acute intoxication with soman and Russian VX (RVX), and to determine critical terms in development of intoxication and diagnostic significance of the biochemical parameters. Activity of blood cholinesterases, the level of aminotransferases and gamma-glutamyltransferase were the biochemical markers during the 1st week after intoxication. Esterase activities of albumin and PON1 were also estimated. The level of creatinine and urea were the non-specific markers at late stages (4-6 weeks). Parameters of hemolytic durability of red blood cells were found to be non-specific characteristics to estimating the pathogenesis at medium and late terms (2 and 4 weeks). Parameters of plasma hemostasis (APTT, PT, fibrinogen, ADAMTS13, VWF) characterize development of acute intoxication at early stages (1 week), though some of them (PT, ADAMTS13, VWF) could characterize intoxication development at medium terms (2 weeks). Dynamics of biochemical and hemostatic parameters investigated indicate that the critical terms under acute intoxication with soman and RVX are the first day (the risk of hemorrhage and DIC syndrome) and 2 weeks (the risk of thrombotic complications). The risk of thrombotic complications appears to be more expressed under acute intoxication with soman.

PS 2048 PROTECTION AGAINST ACHE - INHIBITION.

I. Petrikovics[1], R. J. Kern[2], J. R. Wild[2], G. Kuzmitcheva[1] and M. E. Wales[2]. [1]*Chemistry, Sam Houston State University, Huntsville, TX and* [2]*Biochemistry, Biophysics, Texas A&M University, College Station, TX.*

Currently, the standard treatment for OP poisoning is the co-administration of the AChE reactivator pralidoxime (2-PAM) and the anticholinergic atropine. Although 2-PAM + atropine combination therapy can effectively treat the symptoms of OP poisoning, this is accomplished without protecting AChE from subsequent inhibition by the OP persistent in the body. This study describes the in vitro and in vivo efficacy of OPAA and OPH, when nano-intercalated with a dendritic poly(2-alkyloxazoline) polymer (DP OPAA and DP-OPH), in decreasing the AChE inhibition by the model OP molecules, DFP and paraoxon, respectively. Reactivation of the OP-inhibited AChE was proportional to the pralidoxime concentration, and the AChE level was proportional to the concentrations of DFP or paraoxon and the DP-OPAA or DP-OPH, respectively. 2-PAM reactivates the DFP inhibited AChE, with a reactivation rate of approximately 58 units of AChE per μM 2-PAM. In the presence of 18 mM DFP, AChE was inhibited greater than 95%. When 2-PAM was employed with DFP, the AChE activity was still inhibited up to 70%. The application of DP-OPAA further reduced the DFP-mediated inhibition of AChE to approximately 44%. Similar experiments with paraoxon indicated that 0.7 nM paraoxon completely inhibited 0.12 U of AChE. The application of 2-PAM alone limited the inhibition to 90%, while the 2-PAM + DP-OPH combination decreased the inhibition to approximately 70%. Measuring the in vivo plasma cholinesterase level in mice after administering sub-lethal doses of DFP alone, and in conjunction with 2-PAM and 2-PAM + DP-OPAA indicated that the inhibition was significantly less when 2-PAM was employed alone, or in a combination with DP-OPAA. Similar effects on AChE activity were observed when paraoxon was employed with and without (DP-OPH) and/or 2-PAM. Since OP compounds are cholinesterase inhibitors, monitoring the AChE level in biologic fluids can be utilized as an indicator of efficacy of different OP antidotal systems.

PS 2049 REACTIVATION OF PHOSPHORYLATED ACETYLCHOLINESTERASE AND NEURAL PROTECTION IN THE CENTRAL NERVOUS SYSTEM USING NOVEL PYRIDINIUM OXIMES.

R. Pringle[1], E. Meek[1], H. Chambers[1], J. Gearhart[2] and J. Chambers[1]. [1]*Mississippi State University, Mississippi State, MS and* [2]*AFRL, Wright-Patterson AFB, OH.*

Organophosphate (OP) poisoning leads to the inhibition of acetylcholinesterase (AChE) and is traditionally treated using atropine sulfate and an oxime, such as 2-PAM in the U.S. However, the currently available oximes have limited abilities to cross the blood-brain barrier (BBB), which results in little, if any, reactivation of brain AChE. This lack of reactivation in the central nervous system leaves the brain vulnerable to OP-induced neural damage that may have detrimental long-term effects. This study utilized highly relevant sarin and VX surrogates to test the efficacy of novel pyridinium oximes, created to incorporate moieties which increase lipophilicity and BBB penetration. Levels of glial fibrillary acidic protein (GFAP), detected using immunohistochemistry, were measured in the brain as an indicator of neural damage. Adult rats were treated ip with a sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP; 70-80% brain AChE inhibition), followed at 1 hour by im administration of 2-PAM or selected oxime (in addition to appropriate control groups). Upon observation, the NIMP-treated rats displayed seizure activity and they displayed GFAP accumulation compared to controls. As expected, 2-PAM did not reactivate brain AChE or stop seizures. A few novel oximes yielded 10-35% reactivation of brain AChE. Treatment with one of the most effective novel AChE reactivators (NIMP+oxime) attenuated seizures and reduced GFAP levels over NIMP treatment to levels near those of control animals. These results indicate the therapeutic possibilities of these oximes toward reactivation of OP-inhibited brain AChE and the potential to protect the brain from neural damage. Supported by Defense Threat Reduction Agency: 1.E0056_08_AHB_C

PS 2050 IMPROVED DECORPORATION OF THE ACTINIDE RADIOELEMENT ^{241}Am WITH A NOVEL ORALLY AVAILABLE FORMULATION OF DTPA.

G. N. Shankar[1], W. Weber[2], M. Doyle-Eisele[2], N. Bejugam[1], S. Mutyam[1] and R. A. Guilmette[2]. [1]*Pharmaceutical Sciences, SRI International, Menlo Park, CA and* [2]*Center for Countermeasures Against Radiation, Lovelace Respiratory Research Institute, Albuquerque, NM.*

A radioactive byproduct of nuclear fission, Americium 241 (^{241}Am) is present in spent power reactor fuel and nuclear weapons, and is a recognized candidate for use in radiological dispersal devices. The drug of choice for removing ^{241}Am from a contaminated person (decorporation) is diethylenetriamine pentaacetic acid (DTPA). Because DTPA has poor oral bioavailability, the current methods of drug delivery are IV or solution nebulization; this project is developing orally bioavailable formulations of DTPA for decorporating ^{241}Am. In our current efficacy study, we evaluated rat decorporation of a novel tablet formulation of the Ca chelate form of DTPA (SRI-DTPA). Pilot batches of SRI-DTPA were made by compression, under optimized conditions. The optimized tablet formulation included a liquid permeation enhancer known to improve oral bioavailability. The efficacy of SRI-DTPA was tested in groups of 6 young adult F344 rats injected intravenously with a soluble ^{241}Am-citrate complex. A single dose of SRI-DTPA (60 mg) was given by gavage at 1 hr, 1, 5, or 14 days after the ^{241}Am injection. Each rat was placed in a metabolism cage to enable complete, separate collection of urine and feces, and the treated animals were euthanized 7 days after receiving SRI-DTPA (8, 12, or 21 days after ^{241}Am injection).

SRI-DTPA was shown to significantly increase the excretion of ^{241}Am for all treatment times; statistically significant decreases in liver and bone content of ^{241}Am were also observed. For example, for SRI-DTPA treatment at 1 hr, the total body content of ^{241}Am was reduced to 39% of the injected dose, compared with 74% in the untreated controls. We concluded that this formulation of DTPA is very effective in decorporating ^{241}Am in rats, even with a single-dose administration. This work was funded by the NIAID contracts HHSN266200500043C to the University of Maryland School of Medicine, and HHSN26620050047C to SRI International.

PS 2051 EFFECTS OF COPPER DOPED TITANIUM DIOXIDE NANOPARTICLES *IN VIVO*: ROLE OF SOLUBLE METAL.

B. L. Serke[1], N. Corson[1], P. Mercer[1], R. Gelein[1], M. Sahu[2], P. Biswas[2], G. Oberdörster[1] and A. Elder[1]. [1]*Environmental Medicine, University of Rochester, Rochester, NY and* [2]*Energy, Environmental and Chemical Engineering, Washington University, St. Louis, St. Louis, MO.*

The production and use of nanoparticles (NPs; <100nm) in medicine, consumer products, the semiconductor industry and alternative energy, has led to concerns regarding occupational and consumer safety. Cu doped TiO_2 NPs are used to enhance the photocatalytic properties of solar cells, but the literature lacks reports of the toxicological effects of these NPs in the respiratory tract. Moreover, the role of soluble metals that get released upon interactions of NPs in extracellular fluids and within cells is unclear. We hypothesized that Cu doped TiO_2 NPs elicit oxidative stress and inflammation through released soluble Cu. A series of 100% anatase TiO_2 NPs (25-35 nm) was made with increasing amounts of Cu doping. In comparison to metallic Cu NPs (60nm), which produced 4.76 μM H_2O_2 equivalents per μg in an acellular reactive oxygen species (ROS) assay, Cu doped TiO_2 (7%), CuO, and 100% anatase TiO_2 ROS levels were undetectable. Dynamic dissolution of the NPs in simulated lung lining fluid (pH=7.4) showed that there was substantial Cu release over 30 hours (Cu, 95%; 7% Cu doped TiO_2, 88%; CuO, 72% of starting Cu mass). Following intratracheal instillation (24 hrs) in male F344 rats (0-200 μg), the greatest increases in percentage of bronchoalveolar lavage fluid (BALF) neutrophils were found for metallic Cu and CuO NPs as compared to saline controls. Although the Cu in the doped TiO_2 was bioavailable, the overall amount released was lower, as was the inflammatory response. Similar trends were found for changes in BALF lactate dehydrogenase activity and protein concentration. These results suggest that the inflammatory potential of Cu doped TiO_2 NPs is related to metal solubilization in the respiratory tract following exposure. This research was funded by NIH R01CA134218, P30ES01247 and T32ES07026.

PS 2052 GENE EXPRESSION PROFILING OF IMMUNE-COMPETENT CELLS EXPOSED TO METAL OXIDE NANOPARTICLES.

B. Fadeel[1], S. Tuomela[2], R. Autio[3], A. Kunzmann[1], B. Andersson[4], J. Shi[1], T. Bürki-Thurnherr[5], P. Wick[5], O. Arslan[6], S. Mathur[6], A. Scheynius[4], H. Krug[5] and R. Lahesmaa[2]. [1]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden,* [2]*University of Turku and Åbo Akademi University, Turku Centre for Biotechnology, Turku, Finland,* [3]*Department of Signal Processing, Tampere University of Technology, Tampere, Finland,* [4]*Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden,* [5]*Laboratory for Materials-Biology Interactions, Swiss Federal Laboratories for Material Science and Technology, St. Gallen, Switzerland and* [6]*Inorganic and Materials Chemistry, University of Cologne, Cologne, Germany.*

Engineered nanomaterials exhibit novel physicochemical properties that determine their interaction with biological systems. However, there is a paucity of data on the interaction of nanoparticles with the immune system. In the present study, we performed a comprehensive in vitro assessment of two common metal oxide nanoparticles, TiO2, and ZnO using human monocyte-derived macrophages (HMDM) and monocyte-derived dendritic cells (MDDC), and a human T cell leukemia-derived cell line (Jurkat). We observed that ZnO but not TiO2 nanoparticles induced a dose-dependent toxicity and ROS induction in all three cell types. Subsequently, we attempted to determine the signaling pathways underlying the cytotoxic response by global gene expression profiling using Illumina's Sentrix® HumanHT-12 Expression BeadChips. In brief, 2703 genes were significantly differentially expressed in HMDM upon exposure to ZnO nanoparticles compared to control conditions, while in MDDCs only 12 genes were significantly differentially expressed. In Jurkat cells, 980 genes were significantly differentially expressed. It is noteworthy that the gene expression of different me-tallothioneins was significantly upregulated in all three cell types. Overall, these studies demonstrate the feasibility of assessing nanoparticle responses using transcriptomics approaches. We plan to study additional classes of engineered nanoparticles to explore whether common "nanotoxicogeno-mic" signatures exist.
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PS 2053 UPTAKE OF SILICA NANOPARTICLES AND QUANTUM DOTS IN DIFFERENTIATED AND UNDIFFERENTIATED THP-1 CELLS: AN ILLUSTRATION OF CHALLENGES IN MEASURING NANOPARTICLE UPTAKE BY CELLS.

G. K. Hinkley[1], D. Barber[1], P. Sharma[2], K. Powers[2] and S. Roberts[1]. [1]*Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL and* [2]*Particle Engineering Research Center, University of Florida, Gainesville, FL.*

Uptake of nanoparticles by cells can be important in producing biological effects. Nanoparticle properties that influence cellular uptake remain poorly understood. In this study, uptake of dye-doped silica nanoparticles and quantum dots were evaluated in differentiated (macrophages) and undifferentiated (monocytes) human THP-1 cells. Cells were differentiated by treatment with 200nm phorbol 12-myristate 13-acetate (PMA) for 24 hours. Silica particles (ca. 50 nm) with negative (-45mV at pH 7) and positive (+20mV at pH 7) zeta potentials were tested, along with carboxy- and aminated/PEG-coated quantum dots. Cells were dosed with silica particles at a concentration of approximately 1mg/ml, and with quantum dots at a concentration of 3nM. Cell uptake of nanoparticles was assessed using fluorescent microscopy, flow cytometry and confocal laser scanning microscopy. In undifferentiated cells, fluorescent microscopy and flow cytometry suggested extensive uptake of particles (>90% fluorescent cells within 1 hr for both -45mV silica particles and carboxy- quantum dots), but confocal microscopy showed that nanoparticles were bound to the plasma membrane and not taken up by the cells. The fluorescent and flow cytometry results were similar for differentiated THP-1 cells (>90% fluorescent cells within 1 hr for both -45mV silica particles and carboxy-quantum dots); however, confocal microscopy demonstrated that nanoparticles were in fact taken up by the cells. This study provides an example of a situation in which plasma membrane binding of nanoparticles did not lead to uptake (in the monocytes). Methods that do not effectively distinguish between plasma membrane bound nanoparticles and particles internalized within the cell can potentially provide misleading results regarding nanoparticle uptake. [Supported in part by the Center for NanoBio Sensors, University of Florida]

PS 2054 THE TOXIC EFFECTS OF MESOPOROUS SILICA NANOPARTICLES IN MACROPHAGES.

S. Lee[1], H. Yun[2] and S. Kim[1]. [1]*Pharmacology, Kyungpook National University, Daegu, Republic of Korea and* [2]*Engineering Ceramics Research Group, Functional Materials Division, Korea Institute of Materials Science (KIMS), changwon, Republic of Korea.*

Studies on nanoparticles designed for application in biology have grown significantly over the last few decades. However, due to their small size, many nanoparticles can enter cell organelles and disrupt normal cell functions. Thus, there are general concerns regarding potential toxic effects of nanoparticles after administration. Especially, mesoporous silica (MPS) nanoparticles offer advantage in drug delivery systems, tissue engineering, labeling and bioseparation material technologies, and transfection devices. We investigated the toxicity of MPS and underlying mechanism of actions in murine macrophages. MPS significantly lowered cytotoxicity for 1 or 3 days as compared with colloidal silica nanoparticles using MTT assay. We found that apoptotic cell death was reduced on MPS treatment than colloidal silica due to reduction of p38, c-Jun N-terminal kinase (JNK), and caspase 3 activations on MPS. In addition, inflammatory response such as IL-1β, TNF-α, and IL-6 cytokines were also reduced by MPS through inhibition of NF-κB in peritoneal macrophages. These results suggested that MPS nanoparticles has lower toxicity and inflammatory response than colloidal silica by inhibition of p38, c-Jun N-terminal kinase (JNK), caspase 3, and NF-κB.

PS 2055 ZNO NANOPARTICLE NEUROTOXICITY REQUIRES CELLULAR INFLUX OF PARTICLES.

A. M. Klim[1], B. V. Madhukar[2] and P. Cobbett[1]. [1]*Pharmacology and Toxicology, Michigan State University, East Lansing, MI and* [2]*Pediatrics and Human Development, Michigan State University, East Lansing, MI.*

Recent increase in the use of nanoparticles in pharmaceutical and other industries has raised concerns of their potential impact on human health. To assess the potential neurotoxicity of engineered ZnO nanoparticles, their effect was tested on a neuroblastoma (N1E-115) cell line. Toxicity was evaluated using the MTT assay which measures activity of mitochondrial reductase enzymes and is used to measure cell viability. Initial experiments demonstrated significant cell death following cell exposure (24 hours) to ZnO nanoparticle at concentrations greater than 100nM. Similar loss of cell viability was produced following cell exposure to ZnO nanoparticles at 250nM for two hours. In later experiments, it was observed that ZnO

nanoparticle-induced toxicity progressed even after replacing nanoparticle-containing medium with nanoparticle-free medium. We hypothesized that ZnO nanoparticle toxicity requires that nanoparticles enter the cells and that toxicity was not simply due to nanoparticle contact with the cell membrane. To test the hypothesis, we examined ZnO nanoparticle toxicity in conditions which reduce energy-requiring uptake mechanisms including membrane transporters and endocytosis. Nanoparticle toxicity was evaluated at 37 C and 21 C and it was observed that toxicity was reduced during incubation at 21 C. This temperature dependence of nanoparticle toxicity was concluded to be due to reduction of nanoparticle uptake into the cells and not simply due to slowing of intracellular biochemical processes affected by the nanoparticles. AMK was supported by Larry D. Fowler Scholarship from MSU College of Natural Science.

PS 2056 THE USE OF ACCELERATOR MASS SPECTROMETRY TO QUANTIFY THE ADMET PROPERTIES OF SIO2 NANOPARTICLES *IN VIVO.*

M. A. Malfatti[1, 3], E. Kuhn[1, 3], W. Wang[2, 3], S. Retterer[2, 3] and K. Turteltaub[1, 3]. *[1]Lawrence Livermore National Laboratory, Livermore, CA, [2]Oak Ridge National Laboratory, Oak Ridge, TN and [3]Battelle Center for Fundamental and Applied Systems Toxicology (B-FAST), Battelle Memorial Institute, Columbus, OH.*

The ever-increasing use of nanoparticles (NPs) for a wide variety of commercial applications has led to concerns about their biosafety. Silica NPs have been widely developed for biomedical use but their biodistribution and toxicity has not been extensively investigated. To understand their biological fate it is essential to comprehensively determine the absorption, distribution, elimination, and toxic properties of these particles. To better understand the relationship between administered dose, ADME properties, and ultimate toxicity, the ultra sensitive mass spectrometry technique of Accelerator Mass Spectrometry (AMS) was used to determine the pharmacokinetic parameters of 14C-SiO2 NPs in mice. AMS measures isotope ratios with high selectivity, which allows for the quantification of extremely low concentrations of material with high sensitivity and precision. In the current study, 14C-SiO2 NPs were synthesized using amorphous silica. The 14C –label was incorporated into the core of the NPs. Particle size was determined to be 33 nm with a specific activity of 0.23 nCi/mg. Mice were subsequently dosed (IV) with the 14C-SIO2 NPs and blood and tissue were collected at specific time intervals up to 24 hrs. Nanoparticle concentration in blood and tissue was quantified by AMS. Initial results have shown plasma concentration curves from mice dosed with the 14C-SiO2 NPs to have a rapid plasma clearance of the particles with a T1/2 of 21.6 min. Tissue distribution showed an accumulation of particles in the liver and spleen up to 60 min after dosing. These results indicate that AMS is an effective tool to accurately and precisely quantify ADME properties of SiO2 nanoparticles in vivo.
This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344 and supported by Battelle Memorial Institute, CRADA No. PNNL/284.

PS 2057 INHALED CADMIUM OXIDE (CDO) NANOPARTICLES CAUSES LUNG INJURY IN ADULT MALE MICE.

L. K. Rosenblum, J. L. Blum, J. Q. Xiong and J. T. Zelikoff. *Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

The nanotechnology industry is growing rapidly in order to utilize the novel properties of engineered nanoparticles. Nanoparticles are generated for industrial and medical uses, and CdO nanoparticles specifically are used as a starting material for the production of medically important, quantum dots. Inhaled bulk cadmium is known to be toxic, however little is known about its toxic properties at the nanoparticle size. A study was carried out using adult male CD-1 mice to determine the toxicity of inhaled CdO nanoparticles in the lungs. Male mice between 12- and 14-wk-of- age were exposed daily for seven days (3 h/d) to 240 μg CdO/m3 (15.3 ± 0.1nm). Following exposure, mice were sacrificed either 24 hours later or 1 week post-exposure. At the designated time point, bronchioalveolar lavage (BAL) was performed on all mice, and the BAL fluid recovered and used to assess levels of lactate dehydrogenase (LDH), total protein, and matrix metalloproteinase (MMP) protein transcription (by Western blot) and MMP activity using gelatin zymography. Increased levels of lung injury markers, i.e., LDH and protein levels were seen in CdO treated mice for both the 24 hour and 1 week time points. Some decline in protein and LDH levels were observed over time, however at 1 week the levels still remained significantly elevated above control. Inhalation of CdO also significantly increased MMP-2 (1.5-fold above control for 24h and 3.4-fold for 1wk) and MMP-9 activity (above controls); inducible MMP-9 activity was only observed in the CdO treated group. These data suggest that even short-term inhalation exposure to a moderate level of CdO nanoparticles can cause sustained lung tissue damage through multiple injury and repair mechanisms. Supported by NIEHS Grant No. 26B2600.

PS 2058 INHALED NICKEL NANOPARTICLES AND MURINE ENDOTHELIAL PROGENITOR CELLS.

E. N. Liberda, A. K. Cuevas, Q. Qu and C. Lung Chi. *Environmental Medicine, New York University, Tuxedo, NY.*

Introduction: We have previously shown that exposure to inhaled nickel nanoparticles cause reduced number of bone marrow endothelial progenitor cells (EPCs) as well as reduced functions of these cells. These findings have implications in human exposure to particulate matter high in nickel content as well as occupational environments. In order to elucidate how these observed effects in EPCs occur, the gene expression and function of bone marrow EPCs from nickel nanoparticle exposed or filtered air control mice was determined. In addition to the *in vivo* experiments, an *in vitro* exposure to serum collected from previously nickel exposed and control mice was performed to determine if the circulating plasma proteins can cause adverse outcomes in EPC function. **Methods:** Bone marrow EPCs from C57BL/6 mice (n=8) were isolated 30mins and 12hrs after a 5 hr exposure to 459 ug/m3 nickel nanoparticles. After 10 days in culture, EPCs were harvested for their RNA or used in a variety of assays including chemotaxis, tube formation, and viability. Furthermore, blood smears were also performed in order to determine if a circulatory inflammatory state could be observed. Gene expression was assessed using RT-PCR methods for genes associated with EPC homing, release, and adhesion. Similar endpoints were assessed for EPCs cultured with serum from exposed and control mice. **Results and Conclusions:** Differences between control and exposure groups were observed for neutrophil counts in blood. Cells harvested immediately post exposure did not have any differences in viability, however, exposed mouse EPCs harvested 12hrs after exposure showed reduced viability compared to the 30 min group. Furthermore, differences were observed in gene expression and other experiment on EPCs. Lastly, similar differences were observed in EPCs cultured in plasma from exposed and control mice. These results indicate that inhalation exposure to Ni nanoparticles below the current OSHA permissible exposure limit for Ni compounds could initiate alterations in bone marrow progenitor cells leading to the development of cardiovascular disease.

PS 2059 GOLD NANOSPHERES CAUSE SIZE-DEPENDENT CELLULAR ALTERATIONS IN HUMAN KERATINOCYTES.

C. M. Garrett[1, 2], A. M. Schrand[2] and S. M. Hussain[2]. *[1]Air Force Research Laboratory, Henry M. Jackson, Wright-Patterson Air Force Base, OH and [2]Applied Biotechnology Branch, Wright-Patterson Air Force Base, Dayton, OH.*

The unique properties of gold nanoparticles (Au NPs) due to their small size, plasmonic nature and relative biocompatibility, in applications such as nanodiagnostics, imaging, photo thermal cancer therapy and targeted drug delivery is revolutionizing the field of medicine. However, understanding the potential molecular effects after low dose nanoparticle exposure remains poorly understood. In order to assess the bio-effects of sub-toxic concentrations of gold nanoparticles (Au-NP) on human keratinocytes, whole-genome expression analysis was performed. Using Human Genome microarrays, we show that human keratinocytes differentially express 368 genes when exposed to AuNP. Ingenuity® Pathway Analysis revealed that the genes were involved in cell-mediated immune responses, cellular development, organization and maintenance, and stress response. Moreover, there are size-dependent differences in the gene expression and subsequent protein expression profiles of these materials. The smallest particles tested (10nm) induced primarily upregulation of genes involved in stress and inflammatory responses (S100A9, CD44, EPPK1), and signal transduction (RASAL2). In contrast, the largest particles tested (60nm) resulted mainly in the down regulation of genes involved in maintaining cell stability (DCN, SCAMP1), cell signaling (EPHA4) and cell trafficking (SCAMP1). The intracellular uptake of all of the different sized Au NPs was demonstrated via TEM and ultrahigh resolution light microscopy. In summary, the results demonstrate that size dependant uptake of Au NPs altered some of the key genes involved in inflammation, stress response and cellular development.

PS 2060 *IN VITRO* MECHANISTIC STUDY ON THE TOXICITY OF COMMERCIAL ZINC OXIDE NANOPARTICLES.

T. Bürki-Thurnherr[1], L. Diener[1], C. Waldmann[2], O. Arslan[2], X. Maeder-Althaus[1], P. Wick[1], S. Mathur[2] and H. F. Krug[1]. *[1]Laboratory for Materials-Biology Interactions, EMPA - Swiss Federal Laboratories for Materials Testing and Research, St. Gallen, Switzerland and [2]Inorganic and Materials Chemistry, University of Cologne, Cologne, Germany.* Sponsor: B. Fadeel.

Metal oxide nanoparticles exhibit unique properties which are exploited in many fields of technology and medicine. Among these novel materials ZnO is already produced in high tonnage, and their intentional use in commercial applications such as for antibacterial coating or UV absorber in sunscreens and textiles requires immediate knowledge on their toxic potential. The interaction of nanomaterials

with immunocompetent cells is of special interest as these cells are present throughout the body and are crucial for the efficient recognition and elimination of pathogens and foreign materials. Here, we investigated the adverse effects of large-scale produced ZnO nanoparticles on a human T cell leukemia-derived cell line (Jurkat) and the underlying mechanisms. Already low concentrations of ZnO nanoparticles induced major cell death including apoptotic and late apoptotic/necrotic cells. Using mutant cell lines deficient in various components of apoptotic signaling pathways we found that ZnO nanoparticles activate none of the classical apoptotic mechanisms, namely extrinsic or mitochondrial apoptosis pathways. However, reactive oxygen species are involved as the antioxidant agent N-acetylcystein significantly reduced ZnO-induced apoptosis. No ZnO nanoparticles were observed in treated cells but instead rapid dissolution and high concentrations of Zn-ions were found both extra- and intracellularly. Removal of these ions in the medium and/or within the cells completely inhibited ZnO-induced apoptosis. To conclude, our study indicates that ZnO nanoparticle toxicity in Jurkat T cells is predominantly mediated by Zn-ions and involves oxidative stress signaling mechanisms but not the classical apoptotic pathways. Sponsored by the Seventh Framework Programme of the European Commission (FP7-NANOMMUNE, grant no. 214281)

PS 2061 MACROPHAGE ACTIVATION AND MIGRATION ON TRANSPARENT TITANIUM NANOSTRUCTURE.

D. Khang[2], S. Lee[1] and S. Kim[1]. [1]*Pharmacology, Kyungpook National University, Daegu, Republic of Korea and* [2]*Center for Nano-Morphic Biological Energy and School of Nano and Advanced Materials Science and Engineering, Gyeongsang National University, Jinju, Republic of Korea.*

The immunotoxicity of implanted nanostructured titanium is a paramount issue for vascular, dental and orthopedic applications. Here we report a deactivation of macrophage immune response on nanostructured titanium surfaces. Basically, NO and iNOS were significantly reduced on nano featured surfaces as compared with flat. For examining cytoskeleton variation of macrophage, both confocal microscopy and live cell recording system were used. Importantly, results indicated that nano-bump-surfaces features inhibit the migration activity, such as migration distance and migration speed within 12 hours. In addition, through the live cell recording analysis, we found that level of activated macrophage on nanomaterials were recovered within 24 hours, compared to flat titanium surfaces, after the initial adhesion of macrophage on biomaterials. In western blot experiment, focal adhesion kinase (FAK) and p-FAK, showed significant difference of cytoskeleton morphology of macrophage at 4 hours. In conclusion, this study demonstrated the migration of macrophage on nanomaterials was determined by nano surface topography which led to quite different behavior (immunological aspect) compared to flat surfaces in identical chemistry.

PS 2062 ADHESIVE PROPERTIES OF HUMAN ENDOTHELIAL CELLS EXPOSED TO AMORPHOUS SILICA NANOPARTICLES.

D. H. Napierska[1], R. Quarck[2], L. Thomassen[3], L. Gonzalez[4], V. Rabolli[5], D. Lison[5], M. Kirsch-Volders[4], J. Martens[3], M. Delcroix[2], B. Nemery[1] and P. H. Hoet[1]. [1]*Research Unit for Lung Toxicology, K.U. Leuven, Leuven, Belgium,* [2]*Pulmonary Circulation Unit, K.U. Leuven, Leuven, Belgium,* [3]*Center for Surface Chemistry & Catalysis, K.U. Leuven, Leuven, Belgium,* [4]*Free University of Brussels, Brussels, Belgium and* [5]*Catholic University of Louvain, Brussels, Belgium.*

The present study was undertaken to examine the effect of amorphous (monodisperse) silica nanoparticles (SNP) of different sizes on endothelial cell function, in the absence or presence of a previously established in vitro human airway model consisting of triple cell co-cultures. An immortalized human endothelial cell line (EA.hy926) and primary human pulmonary artery endothelial cells (hPAEC) were seeded in inserts (apical compartment) which were introduced or not above triple cell co-cultures in the basolateral compartment (pneumocytes (A549), macrophages (THP-1), and mast cells (HMC-1)). Endothelial cells (EC) were incubated with silica nanoparticles (hydrodynamic diameter of 28, 59 and 174 nm). The adhesion of monocyte cell line (U937) to EC was assessed, and endothelin-1 (ET-1) levels in culture media were measured. Additionally, the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) was investigated in SNP-exposed EC. Exposure of EA.hy926 cells to 28 and 59 nm particles at non-toxic concentrations induced a significant increase in U937 cell adhesion and decrease in ET-1 secretion (up to 2-fold). Both adhesion and ET-secretion were increased when EC exposure to SNP was performed in the presence of triple cell co-cultures. Exposure to all three SNP induced expression of ICAM-1 but no VCAM-1 in the EA.hy926 cells, and both ICAM-1 and VCAM-1 in hPAEC cultures. Our results suggest that exposure of human endothelial cells to amorphous silica nanoparticles influences adhesive properties of the studied cells. Work was partially financed by the Belgian Ministry of Scientific Policy in the frame of S2NANO project (contract number SD/HE/02A).

PS 2063 CYTOTOXICITY OF SILVER NANOPARTICLES ON JURKAT T CELLS.

H. Eom and J. Choi. *University of Seoul, Seoul, Republic of Korea.* Sponsor: D. Ryu.

In this study, to identify potential harmful effect of silver nanoparticles (AgNPs) on human health, toxicity assay was conducted using human cells derived from various organs, such as, Jurkat T, NCI-H460, HeLa, HepG2, MCF-7, and Beas-2B. Among tested cell lines, Jurkat T cell shows a dramatically high sensitivity to AgNPs exposure than to Ag ion. Therefore, mRNA and microRNA (miRNA) microarray analysis was conducted on Jurkat T cells. Microarray results indicate that more differentially expressed genes and miRNAs were induced by AgNPs than by Ag ion and AgNPs induced gene expression were not clustered with control and Ag ion induced ones. This may explain higher sensitivity of Jurkat T cell to AgNPs than to Ag ion and also suggest toxicity of AgNPs took a distinct pathway from that of Ag ion. Based on the results on microarrays and our previous study, oxidative stress was thoroughly analyzed as mechanism of toxicity of AgNPs. Taken overall results on oxidative stress parameters together, AgNPs exposure produce reactive oxygen species (ROS) in Jurkat T cells, which activates p38 mitogen-activated protein kinase (MAPK) through nuclear factor-E2-related factor-2 (Nrf-2) and nuclear factor-kappaB (NF-κB) signaling pathway and further induce DNA damage, cell cycle arrest and apoptosis. In conclusion, our result suggests AgNPs may induce significant and selective toxicity on AgNPs on Jurkat T cell, including genotoxicity, therefore rigorous toxicity evaluation and risk assessment should be conducted using various different cell types and biological systems, prior to widespread use of AgNPs. Acknowledgments : This work was supported and by the Mid-career Researcher Program through National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0027722) and by the Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2010-0016195)

PS 2064 TOXICOLOGICAL INVESTIGATION OF SILVER NANOPARTICLES ON MAPK PATHWAYS AND RELATED TRANSCRIPTION FACTORS IN *CAENOHABDITIS ELEGANS.*

J. Roh, D. Lim, H. Eom, J. An and J. Choi. *University of Seoul, Seoul, Republic of Korea.* Sponsor: D. Ryu.

In our previous study, the functional genomics analysis was conducted for investigation of toxicity of silver nanoparticles in the nematode, *Caenohabditis elegans*; the results suggests that oxidative stress seems to be an important mechanism of AgNPs toxicity in *C. elegans* and *pmk-1*, p38 MAPK plays an important role in defense process towards oxidative stress induced by AgNPs in *C. elegans.* In this study, to identify *pmk-1* dependant transcription factors and downstream genes, the response of stress-response transcription factors (i.e. *daf-16, cep, skn-1, hif-1*) and that of potentially stress response genes (i.e. *gst-4, cyp35a2, sod-3* and *mtl-2*) was investigated in presence of AgNPs in wildtype and *pmk-1*(*km25*) mutants using gene expression, ROS formation and reproduction, as endpoints. Among tested transcription factors, *hif-1* seems to show *pmk-1* dependant response. An increase in the expression of *gst-4* genes was observed in wildtype exposed to AgNPs, but which was not observed in the *pmk-1*(*km25*) mutant, similar tendency was observed in *mtl-2* gene expression, which suggests that *gst-4* and *mtl-2* genes expression appears to be dependent on *p38* activation. The high expressions of the *sod-3* gene in AgNPs exposed *pmk-1* (*km25*) mutant can be interpreted as compensatory mechanisms in the absence of important stress response genes.
Keywords: *Caenorhabditis elegans*; silver nanoparticles; *pmk-1*, p38 mitogen activated protein kinase; *hif-1*; *gst-2*; *mtl-2*
Acknowledgments : This work was supported by the Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2010-0016195) and by the " The Eco-technopia 21 project" of Korean Ministry of Environment.

PS 2065 BIODISTRIBUTION AND PERSISTENCE OF FOUR SIZES OF CERIA ENGINEERED NANOMATERIALS AFTER INTRAVENOUS ADMINISTRATION.

M. Dan[1], P. Wu[2], M. T. Tseng[3], U. M. Graham[2], R. L. Florence[2], J. M. Unrine[2], E. A. Grulke[2] and R. A. Yokel[1]. [1]*Pharmaceutical Sciences, University of Kentucky, Lexington, KY,* [2]*University of Kentucky, Lexington, KY and* [3]*University of Louisville, Louisville, KY.*

Objectives: To test the hypothesis that the size of engineered nanomaterials (ENMs) affects their biodistribution and persistence. Methods: Aqueous dispersions of ~ 5-, 15-, 30- and 65-nm ceria ENMs, synthesized and characterized in-

house, were iv infused into rats over 1 h. Rats were terminated 1 h, 20 h or 30 days later. Cerium in brain, liver, spleen and blood was assessed by ICP-MS and light and electron microscopy. Results were normalized to an infusion dose of 100 mg ceria/kg. Results: Brain cerium at all 3 times and after all 4 ENMs was less than 0.02% of the dose. Liver and spleen had the highest cerium concentrations after infusion of all four ceria ENMs. The spleen weights of 5- and 65-nm ceria ENM-treated rats were significantly greater at 20 h and 30 days than controls. For 5 nm ceria, blood cerium decreased dramatically from 1 to 20 h. Liver and spleen cerium increased 231% and 200% from 1 to 20 h, then decreased only 13 and 17% by day 30. After 30 nm ceria infusion, 33% of the dose was in the liver after 20 h and after 30 days. The mass amount of cerium in the spleen increased 3% from 20 h to 30 days, because the spleen weight increased significantly, resulting in 11% of the dose at 30 days. Cerium was significantly lower in the spleen after infusion of 65 nm ceria compared to the 5 nm and 30 nm ENMs. Histopathology was seen in the spleen and liver, including hepatic granuloma. Conclusions: The majority of the cerium from four sizes of ceria ENMs was in the liver and spleen. Thirty nm ceria ENM, which was more persistent in the liver and spleen, was reported as the optimal size to interact with cell membranes. These results show the importance of understanding the distribution and persistence of different sizes of ENMs. Investigation of the influence of size on the fate of ceria ENM in blood and its cell internalization is needed to interpret our results.
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PS 2066 ENDOTHELIN-1 SECRETION IS INHIBITED *IN VITRO* BY TIO_2 NANOPARTICLES.

E. Alfaro-Moreno[1], A. Montiel-Dávalos[1], D. Napierska[2] and R. Quarck[3]. *[1]Investigacion Basica, Instituto Nacional de Cancerologia, Mexico, Distrito Federal, Mexico, [2]Lung Toxicology Unit, K.U. Leuven, Leuven, Belgium and [3]Pneumology Section, K.U. Leuven, Leuven, Belgium.*

Titanium dioxide nanoparticles (TiO_2-NPs) are widely used in the production of cosmetics, surface coatings and the environmental decontamination of air, soil and water. Nevertheless, the toxicological properties of TiO_2-NPs are not fully evaluated, and little is known about the systemic effects of TiO_2-NPs due to inhalation. Endothelial cells could be a target for these particles, considering that nanomaterials may translocate from the alveoli. Endothelin-1 plays a major role in endothelium homeostasis and some authors have suggested that particulate matter could induce the expression of this molecule. The aim of this study is to evaluate the effect of TiO_2-NPs in the secretion of endothelin-1 in different strands of endothelial cells. For this purpose, endothelin-1 secretion was detected by chemoluminiscent assay after exposing commercial primary human pulmonary artery endothelial cells (hPAEC), EAHY926 cells or proximal pulmonary artery endothelial cells (PAEC) obtained in our lab. The cells were exposed during 24 h to 0.1, 1, 5 or 10 $\mu g/cm^2$ of TiO_2-NPs. TNF-α was used as a positive control. In some experiments, a triculture of pneumocytes-macrophages-mast cells were exposed to TiO_2-NPs during 24 h and an insert containing endothelial cells was added for another 24 h. Our results showed that TiO_2-NPs significantly inhibit the secretion of ET-1 in hPAEC, EAHY926 and PAEC cells exposed, in a concentration-dependent manner. However, TiO_2-NPs induced the strongest effect on the PAEC. When a triculture was exposed, no changes in the ET-1 secretion were observed in the endothelial cells compartment. In conclusion, TiO_2-NPs inhibits the secretion of ET-1 on all the endothelial cells evaluated, when the particles are in direct contact with the endothelial cells, but no changes are evident due to the communication between different compartments.

PS 2067 BIOAVAILABILITY AND TISSUE DISTRIBUTION OF SILVER NANOPARTICLES IN SPRAGUE-DAWLEY RATS.

M. D. Boudreau[1], C. R. Cozart[1], P. H. Siitonen[1], M. V. Pogribna[1] and N. J. Walker[2]. *[1]Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. FDA, Jefferson, AR and [2]National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

Silver nanoparticles (Ag-NPs) are highly effective antibacterial agents, and this property of Ag-NP is exploited in an expanding number of commercial and consumer product applications. While human exposure to Ag-NPs continues to increase with every new application, public concern grows with regard to their safety. Unfortunately, there is a paucity of research to address the health risks associated with Ag-NP exposure. In this study we examined the effects Ag-NP size and dose on bioavailability, tissue distribution, and clearance in rats. Male and female rats were administered 10, 75, or 107 nm citrate-capped Ag-NP spheres or silver acetate (AgOAc) at 3 or 10 mg/kg body weight via saphenous vein or by gavage, and blood (~ 200 µl) was collected at specified time points over a 72 h period. In separate studies, rats were administered the vehicle (2 mM sodium citrate), Ag-NPs (10, 75, or 107 nm), or AgOAc at 10 or 20 mg/kg body weight by gavage, and 24-h urine and feces were collected for 7 days. Blood, urine, feces, and tissues were analyzed for total silver content by ICP-MS. The shapes of the blood concentration versus time curves of AgOAc or the AgNPs were quite similar; however, the AUC was highest for AgOAc and decreased as the Ag-NP size increased. The AUC and Cmax were lower in males than females. Maximum blood concentrations after oral administration were attained at 12 h irrespective of dose or source of Ag. Tissue distribution of Ag was highest in the liver and kidney, following i.v. and oral administration, respectively. Urinary Ag excretion accounted for only trace amounts of the administered dose. The present results suggest that particle size and sex differences may influence the bioavailability and potentially the distribution of Ag-NPs. Research supported by IAG #224-07-0007 between the FDA and the NTP.

PS 2068 CELLULAR ANTIOXIDANTS AND PREDISPOSITION TO DAMAGE BY METAL OXIDE NANOPARTICLES: A TOXICOLOGICAL COMPARISON BETWEEN HUMAN PULMONARY EPITHELIAL AND MESOTHELIAL CELL LINES.

D. E. Figueroa[1], J. Berg[2], A. Romoser[2] and C. M. Sayes[1, 2]. *[1]Biomedical Engineering, Texas A&M University, College Station, TX and [2]Toxicology Program, Texas A&M University, College Station, TX.*

Particulates in the nano-size range are increasingly utilized in the development of consumer products. However, with this increase in technology, comes the responsibility to mitigate the risks associated with increased exposure. As oxidative stress is a prevalent premise in the nanotoxicology literature, we attempt to analyze the antioxidant capacity of both human pulmonary epithelial (A549) and human mesothelial (Met-5A) cell lines as a model for detecting particle-induced oxidative damage. We utilize both cell lines to examine the toxicological response to hydrogen peroxide as well as a variety of metal oxide nanoparticles including SiO2 (33 nm) and TiO2 (25 nm). Protein expression and/or activity of SOD (Mn & Cu/Zn) and catalase enzymes were measured alongside quantification of the small molecular weight antioxidant glutathione (GSH & GSSG). Cellular viability in response to hydrogen peroxide exposure was measured using both a fluorescence-based Live/Dead assay as well as an absorbance-based MTT assay. Densitometric analysis of western blots indicates an increased expression of both Mn-SOD (156%) as well as Cu/Zn SOD (112%) in Met-5A over A549 cells. However, both expression and activity of catalase was shown to be higher in the A549 cells. Similarly, total glutathione levels were higher in A549 cells than Met-5A cell (2.3 µM/10k cells vs. 1.69 µM/10k cells, respectively). Met-5A cells were an order of magnitude more sensitive to peroxide than A549 (LD50 139.9 µM vs. 1.28 mM, respectively). Further work aims to elucidate the toxicological response of these cell lines to metal oxide nanoparticles using biomarkers of oxidative stress as well as apoptosis.

PS 2069 BUILDING MATHEMATICAL MODELS FOR NANOPARTICLE-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION: A STUDY COMPARING SILVER, ZINC, COPPER, NICKEL, AND IRON NANOPARTICLES.

P. A. Smith[1], I. Ivanov[2] and C. M. Sayes[1, 2]. *[1]Biomedical Engineering, Texas A&M University, College Station, TX and [2]Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX.*

As nanomaterials are continually incorporated into a number of consumer and biomedical products, it is becoming more important to develop predictive models that can assess the potential risks associated with nanoparticle exposure to humans. Specifically, nano-sized metal colloids (NMC) have been reported to be excellent catalytic, antimicrobial, or strengthening agents. This study examines five NMC systems, namely silver, zinc, copper, nickel, and iron. We utilized mathematical models to develop nanoparticle "Structure, Activity, Function, and Toxicological (SAFT)" quantitative relationships. Quantitative relationships have been traditionally used to analyze interactions between a material's structure and biological activity. Because the relation between the presence of reactive oxygen species (ROS) and cellular response is well documented in the peer-reviewed literature, we chose to study the relationships between NMC physicochemical characteristics and ROS production. In this study, we analyzed each NMC suspended in ultrapure water and cell culture media. We measured engineered particle size, particle size in suspension, concentration, pH, and zeta potential. Preliminary results show that these NMC have similar physicochemical trends (when comparing size in water vs. size in media), but have different ROS productions. The metals ranked from most productive to least productive are: copper, iron, zinc, nickel, silver. In conclusion, we found that for NMC systems, it is possible to correlate physicochemical properties to potential human health risks.

PS 2070 THE TOXICITY OF SILVER AND SILICA NANOPARTICLES IN COMPARABLE HUMAN AND MOUSE CELL LINES.

R. Foldbjerg[1, 2], C. Beer[1], D. S. Sutherland[2] and H. Autrup[1]. [1]*Environmental and Occupational Medicine, Aarhus University, Aarhus, Denmark and* [2]*Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark.*

The toxicity of silica (SiO2) and PVP-coated silver (Ag) nanoparticles (NPs) was investigated in two pairs of human or mouse cell lines originating from lung epithelium (A549 and ASB-XIV) and macrophages (THP-1 and J744A.1). Both NPs were characterized in H2O and cell media. The primary sizes were 69 nm (Ag) and 27 nm (SiO2) as determined by TEM. Cytotoxicity was tested after 24 h in terms of viability by dehydrogenase activity (WST-8), apoptosis (Annexin V/PI) and the formation of ROS (DCF). Murine cells are more sensitive to NPs than human cells and the toxic response depends on both the NP type and the cell type. The EC50 was significantly higher for SiO2 compared to Ag NP. Significant increases in ROS generally occured at doses close to EC50 or higher leaving the question whether increased ROS were caused by the NPs or as a consequence of cell death. Induction of ROS was also assessed by the comet assay and modifications of DNA. In both human and murine epithelial lung cells, the EC50 NP concentrations from the WST-8 assay correlated well with results from the annexin V/PI assay. Death at EC50 in the lung cells was equally due to apoptosis and necrosis after exposure to either NP. However, large discrepancies were found when comparing EC50 values from the WST-8 assay in macrophages to results from the Annexin V/PI assay. The WST-8 assay appeared to overestimate cell death caused by Ag NPs in J774A.1 cells and by SiO2 NPs in THP-1 cells, whereas the assay underestimated the EC50 values of SiO2 NPs in J774A.1 cells and Ag NPs in THP-1 cells. These discrepancies suggest that the WST-8 assay should be confirmed by additional assays e.g. annexin V/PI. Our preliminary data suggest that NP mediated toxicity can be higher in murine cell lines compared to their human counterparts. This information could be of importance if risk assessment will be based upon animal experimentation. This association will be the subject of investigation in our future work.

PS 2071 AN *IN VITRO* INVESTIGATION OF THE TOXICITY OF SILVER NANOPARTICLES.

P. Sriwichai, F. M. Williams, C. Lye, A. Silmon, A. Groom and E. Mutch. *Medical Faculty, Institute of Cellular Medicine, Newcastle Upon Tyne, Tyne and Wear, United Kingdom.* Sponsor: M. Graham.

During the last decade the public has become increasingly exposed to engineered nanoparticles (NPs), including silver NPs used as anti-microbials, although there is little data on their toxicity or long-term impact on human health. We determined cytotoxicity, genotoxicity and epigenetic changes by silver NPs administered to HL60 and Jurkat cells, which are both derived from human lymphocytes. The cells were selected to investigate the mechanisms of uptake of the NPs since HL60 cells can carry out phagocytosis while Jurkats cannot. HL60 or Jurkat cells were incubated with two types of silver NPs (Nanocomposix) (0.01, 0.1, 0.5, 1 μg/ml) in RPMI medium for 4h, 24h, 48h at 37°C with controls. The silver NPs (10nm diameter) had either positive or negative charges. Cytotoxicity was determined using the MTT assay and DNA damage by the Comet assay. Comet Assay IV software defined the Olive Tail Moment (OTM) of 200 cells. DNA was also extracted from cells exposed to the silver NPs (1μg/ml) to determine global methylation within CpG islands of long interspersed nuclear elements-1 (LINE-1) using pyrosequencing. At minimal levels of cytotoxicity (≤20% loss of cell viability) there were dose related increases in DNA damage by both forms of silver NPs. The profiles of damage with time were generally similar for Jurkat and HL60 cells. There was no significant difference in methylation of LINE-1. These studies show that silver NPs were genotoxic to the cells at concentrations producing low levels of cytotoxicity. However, neither the ability of the cell to carry out phagocytosis nor changes in methylation status influenced the DNA damage seen. Although the underlying mechanisms for the damage are still unclear, the initial charge on the NP when the cells were first dosed did not influence the toxicity.Currently we are investigating whether the charge on the NPs changes during time in culture since preliminary data has indicated that both forms of particles aggregated with time in RPMI.

PS 2072 IRON OXIDE NANOPARTICLES CAUSE OXIDATIVE STRESS AND DERMAL TOXICITY.

A. R. Murray[1, 2], E. Kisin[1], A. O. Inman[3], S. H. Young[1], M. Muhammed[4], T. Burks[4], A. Uheida[4], A. Tkach[1], M. Waltz[2], V. Castranova[1], B. Fadeel[5], J. E. Riviere[3], V. E. Kagan[6], N. A. Monteiro-Riviere[3] and A. A. Shvedova[1, 2]. [1]*PPRB, NIOSH, Morgantown, WV,* [2]*West Virginia University, Morgantown, WV,* [3]*North Carolina State University, Raleigh, NC,* [4]*Royal Institute of Technology, Stockholm, Sweden,* [5]*Karolinska Institute, Stockholm, Sweden and* [6]*University of Pittsburgh, Pittsburgh, PA.*

A number of commercially available metal/metal oxide nanoparticles (NP) such as superparamagnetic iron oxide (SPION) are utilized by the medical field for a variety of applications. We hypothesize that SPION may be toxic to skin via the ability of particles to be internalized and thereby initiate oxidative stress, inducing redox-sensitive transcription factors leading to inflammation. Due to the skin's susceptibility to UV radiation, it is also important to address the combined effect of UVB and NP co-exposure. To test this hypothesis, the effects of dextran-coated-SPION of different sizes (15-50 nm) and manufacturers (MicroMod (MM), Germany and Royal Institute of Technology, Sweden) were evaluated in 2 cell lines: human epidermal keratinocytes (HEK) and murine epidermal cells (JB6 P+). HEK cells exposed to 20 nm (KTH and MM) had a decrease in viability while the 15 and 50 nm particles were not cytotoxic. HEK cells were also capable of internalizing the KTH particles (15 and 20 nm) but not the MM SPION (20 and 50 nm). IL-8 and IL-6 were elevated in HEK cells following exposure to SPION. Exposure of JB6 P+ cells to all SPIONs evaluated resulted in activation of AP-1; however, only UVB plus SPION (15 and 20 nm KTH and 50 nm MM) resulted in significant NF-κB induction in cells. Pre-exposure of JB6 P+ cells to UVB followed by NPs induced a significant depletion of glutathione, release of cytokines, and cell damage as assessed by release of lactate dehydrogenase. These data indicate that co-exposure to UVB and SPIONs was associated with induction of oxidative stress and release of inflammatory mediators. These results verify the need to thoroughly evaluate the adverse effects of UVB when evaluating dermal toxicity of engineered NPs on skin. Supported by NIOSH OH008282, NORA 927Z1LU and EC-FP-7-NANOMMUNE-214281.

PS 2073 A TOXICOGENOMIC ANALYSIS OF GENE EXPRESSION CHANGES IN TIO2 NANOPARTICLE-TREATED MICE.

S. Liu, Y. Yao, W. Yang, X. Ding and J. Gu. *Wadsworth Center, New York State Department of Health, and School of Public Health, State University of New York at Albany, Albany, NY.*

There is a growing concern over the potential toxicity resulting from nanoparticle (NP) exposure. TiO2 NPs, which are widely used in many consumer products, can be absorbed into the body through oral or inhalation exposure. Although TiO2 NPs appear to be relatively safe, recent studies have reported genotoxicity in mice orally exposed to TiO2 NPs. It is believed that inflammation and oxidative stress are the most likely mechanisms underlying NP-induced toxicity; however, the precise molecular responses to TiO2 NP exposure in various tissues remain poorly defined. The aim of the present study was to identify early molecular markers for TiO2 NP exposure and/or toxicity in various mouse tissues, through the use of a toxicogenomics approach, with an ultimate goal of facilitating risk assessment in humans. Adult male and female C57BL/6 mice were exposed to TiO2 NP (<25 nm) in water at a single dose of 500 mg/kg, by oral gavage; control mice received vehicle only. At 24 h post treatment, the mice were sacrificed, and various tissues were collected for analysis. Real-time RNA-PCR quantification of hepatic mRNA expression was performed for a total of 242 genes related to inflammation, oxidative stress, and DNA damage, using a pathway-focused gene expression profiling PCR array (SABiosciences), for detection of early toxicity markers. No histological or functional changes were observed in the liver or other major organs of the treated mice. Interestingly, at least 17 genes were found to be up- or down-regulated in the liver of TiO2 NP-treated mice (with a criterion of >2-fold change, compared to control), including a cytochrome P450 gene (Cyp), which was upregulated by 40-fold at the mRNA level and by 3-fold at the protein level. Furthermore, the upregulation of this Cyp gene appeared to be gender-specific. Additional studies are underway to examine the mechanisms for, and toxicological implications of, the TiO2 NP-induced gene expression changes.

PS 2074 GOLD NANOPARTICLES INCREASE THE LIVER TOXICITY OF ACETAMINOPHEN.

A. M. Keene, A. Knapton, N. R. Hartman, D. Peters, G. Tobin, L. Zhang, S. Stewart, L. Xu, R. Rouse and K. M. Tyner. *Center for Drug Evaluation and Research, U.S. FDA, Laurel, MD.*

Much research has focused on assessing the potential toxicity of nanoparticles when administered alone in various test systems. However, the possibility that nanoparticles may enhance the toxicity of other agents when given simultaneously has not

been explored. This study investigated the effects of acetaminophen (APAP) metabolism and hepatotoxicity in Balb/C mice pretreated with a single dose of citrate-stabilized 10 nm gold nanoparticles (AuNP) (0.5 – 10 mg/kg i.v.). After 72 hours of exposure to AuNP, mice were given a single dose of APAP (30-700 mg/kg i.p.). Blood was collected at multiple time points, and the mice were sacrificed at either 6 or 24 hours following APAP dosing. Multiple assays were used to evaluate liver or serum glutathione concentrations, liver enzyme levels, and APAP metabolites. Preliminary results show an increased mortality associated with a single dose of APAP following a single dose of AuNP. In addition, mice dosed with both AuNP and APAP exhibited increased microscopic liver damage 24 hours after dosing compared to mice that received either agent alone. The observed toxicity is thought to be due at least in part to AuNP depleting glutathione levels, causing a subsequent increase in the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). These results suggest that nanoparticles may contribute to toxicity indirectly by interfering with the response to a secondary insult and reveal the potential importance of additional studies in assessing the safety of nanoparticle-based drugs.

PS 2075 SILVER NANOPARTICLES-INDUCED GENOTOXICITY IN BONE MARROW CELLS OF SPRAGUE-DAWLEY RATS.

A. Patlolla[1] and D. Hackett[1, 2]. [1]*Molecular Toxicology Research Laboratory, Jackson State University, Jackson, MS and* [2]*Biology, Jackson State University, Jackson, MS.*

The antibacterial effect of silver nanoparticles (Ag-NPs) has resulted in their extensive application in health, electronic, and household products. However, while the population exposed to AgNPs continues to increase with ever new applications, silver nanoparticles research remains a controversial with regard to their toxicity to biological systems. In particular, the oral toxicity of AgNPs is of special concern to ensure public and consumer health. Due to the intensive commercial application of AgNPs, their health risk assessment is of great importance. The previous in vitro studies demonstrated that AgNPs caused toxicity in various cell-lines. However, the data on the toxicity of AgNPs in vivo is largely lacking. The aim of this investigation was to determine the clastogenic/genotoxic potential of AgNPs in bone marrow cells of Sprague-Dawley rats; using mitotic index (MI), structural chromosome aberrations (SCA) and micronuclei (MN) formation as genetic endpoints. Four groups of five male rats, each weighing approximately 80 + 2 g, were administered orally, once a day for five days with doses of 5, 25, 50, 100, mg/kg body weight (BW) of AgNPs. A control group was also made of five rats. Chromosome and micronuclei from bone marrow cells were processed and examined following standard protocols. The results demonstrated that AgNPs exposure significantly increases ($p<0.05$) the number of SCA and the frequency of micro-nucleated cells, and decreases the mitotic index in exposed groups compared to control. The results of our study suggest that exposure to AgNPs has the potential to cause genetic damage. Further characterization of their systemic toxicity, genotoxicity and carcinogenicity is also essential.

PS 2076 TOXICOLOGICAL ACTIVITY OF METAL OXIDE NANOPARTICLES UPON BIOLOGICAL MODELS *IN VITRO.*

T. D. Palacios-Hernandez[1, 2], J. L. Garate-Morales[1], F. Cordova-Lozano[2], J. L. Sanchez-Salas[2], C. C. Berry[3], E. Gonzalez-Vergara[1] and M. A. Mendez-Rojas[2]. [1]*Centre of Chemstry and Faculty of Chemical Sciences, Benemerita Universidad Autonoma de Puebla, Puebla, Puebla, Mexico,* [2]*Chemical and Biological Sciences, Universidad de las Americas, Puebla, San Andres Cholula, Puebla, Mexico and* [3]*Centre for Cell Engineering, University of Glasgow, Glasgow, United Kingdom.*
Sponsor: O. Olivero.

The synthesis and characterization of metal-oxide nanoparticles (NPs) modified with low-weight biological molecules and their interaction with biological models in vitro was realized. NPs were synthesized from coordination complexes with Cu2+, Co2+, Zn2+ and carboxylate-containing ligands by thermal decomposition (calcination) and superparamagnetic iron oxide NPs were synthesized from FeCl3 reduced with Na2SO3 in aqueous solution. All the materials were modified on their surfaces with 3-amminopropyltrimethoxysilane to conjugate it with folic acid, L-cysteine, L-tyrosin and glycine by condensation reactions and all the conjugates were marked with fluorescein by the same pathway. All the materials were characterized by spectroscopic techniques (FT-IR, EDS), scanning-transmission electron microscopy (STEM) and mass spectrometry for NPs precursors. The biological interaction of NPs was tested against human fibroblast cultures by Coomasie Blue staining and MTT assay to determine cell morphology and viability. Elemental analysis in all the samples corresponded to the formula ZnO, Fe2O3, CoO and CuO. STEM showed that the materials were aggregated and the size range of NPs obtained by both pathways was between 20-80 nm. The NPs were employed to determine toxicity against Gram positive and negative bacteria (S. aureus and E. coli) by minimum inhibitory concentration (MIC) technique. Due the poor solubility and aggregation the materials tested were toxic for fibroblasts but the aggregation was solved improving some conditions during the synthesis and surface-modification of NPs. The biological activity of metal oxide NPs against bacterial cultures will be discussed later. References: [1] Berry CC, Wells S, Charles S, Curtis ASG. Biomaterials 2003, 24, 4551-4557.

PS 2077 EVALUATION OF GENOTOXICITY OF TITANIUM DIOXIDE NANOPARTICLES USING THE AMES TEST AND *IN VITRO* COMET ASSAY.

J. Yan[1], R. S. Woodruff[2], M. Y. Jones[3], F. Watanabe[4], A. Biris[4] and T. Chen[1]. [1]*Division of Genetic and Molecular Toxicology, U.S. FDA -NCTR, Jefferson, AR,* [2]*Division of Microbiology, Arkansas Regional Laboratory, U.S. FDA, Jefferson, AR,* [3]*Nanotechnology Facility, U.S. FDA -NCTR, Jefferson, AR and* [4]*Nanotechnology Center, University of Arkansas at Little Rock, Little Rock, AR.*

Industrial use of titanium-dioxide (TiO2) nano-material in cosmetics and sunscreen has become commonplace because the rutile form of TiO2 nanoparticles is colorless and photo-active. Therefore, the toxicity of TiO2 nanoparticles needs evaluating due to their wide use. In this study, genotoxicity of TiO2 nanoparticles was assessed using the standard genotoxicity assays, Salmonella reverse mutation assay (Ames test) and the single-cell gel electrophoresis (Comet) assay. X-ray diffraction analysis showed that the TiO2 used in this project is completely anatase. Transmission electron microscope (TEM) and scanning electron microscope (SEM) analysis indicated the nanoparticle size were around 10 x 30 nm. Zetasizer examination displayed that the TiO2 particles aggregated strongly and uniformly in medium, with the aggregate particles sizing around 170 nm. Salmonella strains TA102, TA100, TA1537, TA98 and TA1535 were treated with 8 different concentrations of the nanoparticles, ranging from 38.4 to 4915.2 μg/ml. No mutation induction was found for any of these treatments in the different strains. TK6 cells were treated with 100, 200, 400 and 800 μg/ml of the nanoparticles for 4 hours and the DNA damage was evaluated with the Comet assay. The DNA breakage was significantly increased by every dose group in a dose-dependent manner ($p < 0.05$). The changes of the treatments over the control were 13-fold for 100 μg/ml treatment and up to 22-fold for 800 μg/ml treatment. These results demonstrate that TiO2 nanoparticles are genotoxic in TK6 cells. Also, the data suggest that Comet assay is sensitive to the measurement of genotoxic stress caused by nanoparticles while the Ames test is not.

PS 2078 A COMPARISON OF THE PHOTOTOXIC POTENCY OF SIX TYPES OF TIO2 NANOPARTICLES.

W. K. Boyes[1], K. M. Sanders[1], L. L. Degn[1], R. M. Zucker[1], W. R. Mundy[2], B. Zhao[3] and J. E. Roberts[4, 3]. [1]*Toxicity Assessment Division, U.S. EPA, Research Triangle Park, NC,* [2]*Integrated Systems Toxicology Division, U.S. EPA, Reseach Triangle Park, NC,* [3]*National Institute of Environmental Health Sciences, Research Triangle Park, NC and* [4]*Fordham University, New York, NY.*

Nanoparticles, such as nano-TiO2, are often photoactive and can become phototoxic by the generation of reactive oxygen species (ROS) and free-radical oxidative damage to surrounding tissues. Because the retina is the only part of the central nervous system directly exposed to light, the retinal pigment epithelium (RPE) is an important site of photoreactivity. A human-derived RPE cell line (ARPE-19) was exposed to six types of nano-TiO2 and then observed after exposure to UVA radiation. The TiO2 nanoparticles (anatase, 10 nm, 32 nm, and 200 nm; DeGussa, 27.5 nm; and rutile, 30-40 nm and 200-400 nm) were suspended in DMEM/F12 with 10% fetal bovine serum, sonicated, and characterized by dynamic light scattering. Cells were treated with 0, 0.3, 1, 3, 10, 30, or 100 ug/ml TiO2 in media for 24 hrs and then either exposed to UVA radiation (7.53 J/cm2) or kept in the dark (control condition). Cytotoxicity was assessed after 24 hrs by a live/dead assay (calcein-AM/propidium iodide) and flow cytometry. Live/dead assays indicated that cells exposed to higher concentrations of TiO2 with UVA radiation showed significantly lower viability than dark controls, but the degree of response varied by TiO2 type. The 10 nm anatase and 27.5 nm DeGussa were the most phototoxic compounds tested (LC50 with UVA < 10 ug/ml), while the 200 nm anatase and 200-400 nm rutile were the least phototoxic. Flow cytometry showed that UVA radiation in the presence of TiO2 decreased the cell count, increased cell death, and increased ROS (as measured by Mitosox probe) relative to control cells that had TiO2 but no UVA. These experiments demonstrate that TiO2 nanoparticles become phototoxic when exposed to UVA radiation, with potency dependent on size and/or crystal form. This abstract does not reflect EPA policy

PS 2079 INHIBITORY EFFECTS OF SILVER NANOPARTICLES ON RECOMBINANT CYTOCHROME P450 ENZYMES AND HUMAN LIVER MICROSOMES.

R. Maniratanachote[1], K. Kulthong[1], Y. Kobayashi[2], T. Fukami[2] and T. Yokoi[2]. *[1]National Nanotechnology Center, National Science and Technology Development Agency, Klong Luang, Pathumthani, Thailand and [2]Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Japan.*

Silver nanoparticles have been used in numerous biomedical applications including wound dressing, catheter coating, and bone cement for the purpose of bacterial growth inhibition. Meanwhile, there is an increasing concern in that exposure to these nanoparticles may cause a potential adverse effect to human. Several investigations showed that silver nanoparticles are able to interfere with cellular functions and cause cytotoxicity. In animals, liver is one of the main target organs where silver can be accumulated after exposure. However, there is still lack of data regarding potential effects of silver nanoparticles on cytochrome P450 (CYP), a major drug metabolizing enzyme system in the liver. Changes in CYP activity will be particularly relevant to the toxicity and the therapeutic effect of drugs or xenobiotics. In this study, we investigated effects of silver nanoparticles on CYP inhibition using recombinant CYP enzymes, CYP1A2, CYP2E1, CYP2C9, CYP2D6, and CYP3A4, as well as human liver microsomes (HLM). The specific probe substrates of each CYP isoform were used. The enzyme catalytic activities were measured by HPLC. Physicochemical properties of silver nanoparticles (Sigma 576832) including morphology, size and size distribution, and trace metal contaminants were also analyzed by TEM, DLS, and ICP, respectively. The results showed that silver nanoparticles predominantly inhibited CYP3A4 and CYP2C9, but demonstrated weak affects on CYP1A2 in recombinant CYPs and HLM. For CYP2E1 and CYP2D6, the particles showed stronger inhibitory effects in recombinant CYPs than in HLM. This study suggested that silver nanoparticles can inhibit some of the major CYP isoforms in vitro which might be a useful information for predicting adverse effects of silver nanoparticles in human.

PS 2080 EFFECT OF ORALLY EXPOSURED AMORPHOUS NANOSILICA ON ANTIGEN-SPECIFIC IMMUNE RESPONSES.

T. Yoshida[1, 2], T. Yoshikawa[1, 2], S. Tochigi[1, 2], T. Hirai[1, 2], M. Uji[1, 2], H. Nabeshi[1, 2], K. Nagano[2], Y. Abe[2], H. Kamada[2, 3], S. Tsunoda[2, 3, 4], N. Itoh[1, 2], Y. Yoshioka[1, 2, 3] and Y. Tsutsumi[1, 2, 3]. *[1]Department of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan, [2]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, Osaka, Japan, [3]The Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka, Japan and [4]Department of Biomedical Innovation, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan.*

Ultrafine amorphous nanosilica (nSPs) have already been available as a substitute for larger-size silica particles. However, the biological activity of nSPs remains undefined. Although the biological properties of nSPs may be limited, combined effects by food antigens may enhance food allergy. In the present study, we investigated the effect of repeated oral exposure of nSPs on antigen-specific immune responses in the presence of chicken egg ovalbumin (OVA) as model allergen. nSPs with diameter 30 nm (nSP30) as well as silica with diameter 300 or 1000 nm (nSP300 and mSP1000, respectively) were given orally with 1 mg of OVA. We evaluated in vivo distribution of nSPs by transmission electron microscopy, OVA-specific Immunoglobulin (Ig) E. Results suggested that only nSP30 migrated to blood through gut and reached spleen, although absorption of nSP300 and mSP1000 were not detected, suggesting that nSP30 influence immune system. Next, mice were orally exposed to OVA plus nSPs for four times at weekly interval, and the levels of OVA-specific IgE were determined. We found that oral exposure of OVA plus nSP30 tended to induce higher level of OVA-specific IgE causing type 1 allergy. These results indicate that migration of nSPs below 100 nm to spleen could induce immune-modulating effect. To create safe and effective forms of nSPs, we investigate information about immune response related to physicochemical examination. We believe that these data provide basic information that should help risk analysis of nanomaterials.

PS 2081 MATRIX METALLOPROTEINASES 2 AND 9 AND TISSUE INHIBITORS OF METALLOPROTEINASE 1 IN CERIUM OXIDE INDUCED PULMONARY FIBROSIS.

J. Y. Ma[1], R. R. Mercer[1], M. Barger[1], J. K. Ma[2] and V. Castranova[1]. *[1]PPRB/HELD, NIOSH, Morgantown, WV and [2]School of Pharmacy, West Virginia University, Morgantown, WV.*

Cerium compounds have been used as a diesel engine catalyst to lower the mass of diesel exhaust particles, but are emitted as cerium oxide nanoparticles (CeO_2) in the diesel exhaust. Our previous studies have shown that CeO_2 induced pulmonary inflammation, air/capillary injury, M2 AM differentiation and lung fibrosis. In this study, we investigated the mechanisms involved in CeO_2-induced pulmonary fibrosis. Male Sprague Dawley rats were exposed to CeO_2 (0.15 to 7 mg/kg) by a single intratracheal instillation and sacrificed at 1, 10, 28 and 84 days after exposure. Alveolar macrophages (AM) were isolated by bronchial alveolar lavage (BAL) and the first BAL fluid was saved for further analysis. The activity of osteopontin (OPN), a multifunctional matricellular protein expressed during inflammation and repair, in AM cultured media was significantly increased at 10 and 28 days post exposure. Collagen degradation enzymes, matrix metalloproteinases (MMPs)-2 and -9 and tissue inhibitor of MMP-1 (TIMP-1), in first lavage fluid were markedly induced by CeO_2 at 1 day- and subsequently declined to a lower level at 28 days-post exposure but remained significantly higher than the controls. Hydroxyproline content in lung tissues, a measure for fibrosis, was significantly elevated in the CeO_2-exposed lung tissues in a dose- and time-dependent manner. Morphological analysis showed enhanced collagen fibers in CeO_2 (3.5 mg/kg)-exposed lungs at 28 days post-exposure. In addition, CeO_2 particles were detected in lung tissue and fibroblasts isolated from CeO_2 (3.5 mg/kg)-exposed rats at 28 days after exposure using CytoViva's illumination technology. These results demonstrate that exposure of rats to CeO_2 induced fibrotic lung injury through induction of OPN and the imbalance of MMPs and TIMP in extracellular matrix remodeling. These findings suggest potential health effects of CeO_2 exposure.

PS 2082 PULMONARY TOXICITY OF CRYSTALLINE SILICA AFTER A SINGLE INTRATRACHEAL INSTILLATION IN RATS.

M. Naya[1], N. Kobayashi[1], S. Endoh[2], K. Mizuno[3], R. Nagaosa[1], M. Ema[1] and J. Nakanishi[1]. *[1]Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan, [2]Research Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan and [3]Metrology Institute of Japan, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan.*

Pulmonary toxicity induced by crystalline silica particles, micron and nano size, was assessed in rat intratracheal studies. The average primary size of micron and nano size particles was approximately 2.3 μm and 128 nm, respectively. Groups of male Crl:CD (SD) rats were intratracheally instilled with 5 mg/kg of the crystalline silica particles. Following the instillations, the bronchoalveolar lavage fluid (BALF) biomarkers such as the numbers of white blood cells and neutrophils, levels of lactate dehydrogenase (LDH), protein, and cytokines, and histopathological evaluation of lung, liver, spleen, and cerebrum at 1 week, 4 weeks, 3 months, and 6 months (micron-silica group only) after a single instillation was examined. In all crystalline silica groups, toxicological effects were observed only in the lung but not in the liver, spleen, or cerebrum. In the micron-silica-instilled group, the BALF revealed a significant increase in total cell and neutrophil numbers, LDH and protein concentrations, and TNF-α activities. In the histopathological examination, macrophage accumulation and in inflammatory-cell infiltration in the alveoli, and hypertrophy of the alveolar epithelium cells were observed in the micron-silica group up to 6 months after instillation. In the nano-silica-instilled group, the inflammatory responses were less than that of micron-sized groups at any time points after instillation. These findings indicate that the pulmonary inflammatory responses in the group of micron-silica were severe than that of nano-silica group.

PS 2083 SIZE-DEPENDENT IMMUNE-MODULATING EFFECT OF AMORPHOUS NANOSILICA.

T. Hirai[1, 2], T. Yoshikawa[1, 2], T. Yoshida[1, 2], H. Nabeshi[1, 2], S. Tochigi[1, 2], M. Uji[1, 2], K. Nagano[2], Y. Abe[2], H. Kamada[2, 3], S. Tsunoda[2, 3, 4], N. Itoh[1, 2], Y. Yoshioka[1, 2, 3] and Y. Tsutsumi[1, 2, 3]. *[1]Department of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan, [2]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, Osaka, Japan, [3]The Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka, Japan and [4]Department of Biomedical Innovation, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan.*

Recently, practical uses of Nanomaterials (NMs) are rapidly spreading to a wide variety of fields, such as cosmetics, foods, and medicine. However, potential harmful effects of nanomaterials on humans are raising concerns about their safety, because nanomaterials may possess novel properties different from micro-sized materials. Despite intensive research efforts, relationships between the biological responses and properties of NMs are not well understood. Using amorphous nanosilicas (nSPs) of different sizes, we have systematically investigated the influence of nSP properties on the distribution and immunue-modulating effect. Firstly, we applied silica particles in diameter of 70, 300 and 1000 nm (designated nSP70, nSP300 and mSP1000, respectively) on the ears of BALB/c mice. Transmission electron mi-

croscopic analysis revealed that only nSP70 reached langerhans cells and draining lymph node. Because langerhans cells are sentinels of innate and acquired immunity, nSP-application may have potential immune-modulating effect in vivo. Thus, we next investigated whether the induction of antigen-specific immune responses after intra-dermal injection of the mixture of nSP and model antigen, chicken egg ovalbumin (OVA) by IFN-γ ELISPOT assay. Results indicated that nSPs with a diameter below 100 nm more strongly induce IFN-γ producing cytotoxic T lymphocytes specific for OVA than conventional micro-sized SPs. Our results indicate that the size of NMs as well as the target cell type is critical determinants for the design of safer nSPs.

PS 2084 DISTRIBUTION IN THE TESTIS AND REPRODUCTIVE EFFECTS OF NANOSILICA.

Y. Morishita[1, 2], Y. Yoshioka[1, 2, 3], K. Yamashita[1, 2], K. Higashisaka[1, 2], M. Fujimura[1, 2], H. Pan[1, 2], T. Ogura[1, 2], K. Nagano[2], Y. Abe[2], H. Kamada[2, 3], S. Tsunoda[2, 3, 4], H. Nabeshi[1], N. Itoh[1], T. Yoshikawa[1, 2] and Y. Tsutsumi[1, 2, 3]. *[1]Department of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan, [2]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, Ibaraki, Osaka, Japan, [3]The Center for Advanced Medical Engineering and Informatics, Osaka University, Suita, Osaka, Japan and [4]Department of Biomedical Innovation, Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan.*

Nanomaterials (NMs) are already being applied in universal fields such as electronics, cosmetics, foods, and drugs. However, the increasing use of NMs has raised public concern about their safety. Therefore, it is necessary to investigate the safety of NMs based on biodistribution and biological responses. Especially the reproductive effect of NMs is important issue for preventing the toxicity to offspring. In this study, we examined the effects of nanosilica particles (nSP), one of the most popular NMs, to testis by analyzing the biodistribution to the testis and measuring testicular hormone. We already found that nSP could enter blood circulation by oral, nasal and dermal administrations. Therefore, to assess the biodistribution after entering blood circulation, we analyzed the biodistribution of nSP to the testis after intravenous injection by transmission electron microscopy. nSP with a diameter of 70 nm were detected in sertori cells and spermatocyte, although we did not observe any particles in the testis of larger silica particles-treated mice. Next, we measured the level of testosterone in blood after treatment with nSP via intravenous, oral or nasal administrations. The level of testosterone in nSP-treated mice was different from control mice. These data suggest that nSP could penetrate blood-testis barrier and might induce testicular disorders.

PS 2085 BIODISTRIBUTION AND REPRODUCTIVE TOXICITY OF NANOSILICA FOR ENSURING THE SAFETY OF NANOMATERIALS.

K. Yamashita[1, 2], Y. Yoshioka[1, 2, 3], K. Higashisaka[1, 2], M. Fujimura[1, 2], Y. Morishita[1, 2], H. Pan[1, 2], T. Ogura[1, 2], H. Nabeshi[1], K. Nagano[2], Y. Abe[2], H. Kamada[2, 3], S. Tsunoda[2, 3, 4], N. Itoh[1, 2], T. Yoshikawa[1, 2] and Y. Tsutsumi[1, 2, 3]. *[1]Department of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan, [2]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, Osaka, Japan, [3]The Center for Advanced Medical Engineering and Informatics, Osaka University, Osaka, Japan and [4]Department of Biomedical Innovation, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan.*

Recently, many nanomaterials (NMs) with innovative functions have been developed and already used in several fields. However, knowledge concerning the potential safety of NMs is still fragmentary. Especially, few studies have examined the effect of NMs on maintenance of pregnancy. In this study, we examined the biodistribution and reproductive toxicity of nanosilica particles with different size and surface modification. Pregnant BALB/c mice were treated with silica particles with a diameter of 70, 300 or 1000 nm (nSP70, nSP300 or mSP1000, respectively) via intravenous injection. At first, we examine the biodistribution of silica particles in placenta and fetuses. nSP70 was detected in not only placenta but also fetal liver and fetal brain, although nSP300 and mSP1000 were not detected in these tissues at all. Next, we examined the influence of silica particles on fetuses. nSP70-treated pregnant mice showed the increase of fetal resorption rates and decrease of fetal weights compared to control mice, suggesting that nSP70 induced the miscarriage and fetal growth restriction. In contrast, nSP300 and mSP1000 did not induce these pregnant complications. Furthermore, we showed that nSP70 with functional COOH or NH2 surface modification group did not induce miscarriage and fetal growth restriction. These results suggest that nSP70 induce the miscarriage and fetal growth restriction, and appropriate surface modification suppresses these pregnancy complications. We believe that our findings provide useful information for ensuring the safety of NMs.

PS 2086 INACTIVATION OF ENDOCANNABINOID METABOLISM IN HUMAN THP1 MACROPHAGES FOLLOWING EXPOSURE TO ACTIVATED ORGANOPHOSPHOTHIONATES.

M. K. Ross[1], A. Borazjani[1] and P. M. Potter[2]. *[1]Mississippi State University, Mississippi State, MS and [2]St. Jude Children's Hospital, Memphis, TN.*

Atherosclerosis results from chronic inflammation and dysregulated lipid metabolism. We hypothesize that the endocannabinoid tone of vessel wall macrophages may be perturbed by chronic exposure to activated organophosphothionate insecticides, thus an activated endocannabinoid system may modulate cholesterol metabolism in macrophages. When human THP1 macrophages were cholesterol loaded and subsequently treated with chlorpyrifos oxon (CPO) or paraoxon (PO) (0, 1 and 10μM) for 24h, increasing amounts of the endocannabinoid 2-arachidonoylglycerol (2AG) was secreted. For the 1μM and 10μM CPO treatment groups, amounts of 2AG were 2.5-fold and 8.1-fold higher than the control group, respectively. In contrast, 1μM and 10μM PO resulted in 3.1-fold and 3.5-fold increases in 2AG levels, respectively. This result suggested that THP1 foam cells are capable of 2AG biosynthesis and secretion. It further showed that oxon-mediated inhibition of catabolic enzymes in foam cells contributes to the buildup of 2AG in culture medium in a dose-dependent manner, which is due to inactivation of CES1 and other hydrolytic enzymes. Furthermore, addition of exogenous 2AG to LPS-primed THP1 macrophages, which mimics stimulation of inflamed cells by autacoids such as endocannabinoids, caused significant amounts of prostaglandins (PGF2α and PGE2) to be secreted compared to LPS-primed cells that did not receive 2AG (10-40-fold higher). However, enhanced production of PGs was markedly attenuated 2-4-fold by addition of a CES1 small molecule inhibitor. This result indicated that PG formation by THP1 macrophages is mainly regulated by 2AG hydrolysis since the pool of arachidonic acid produced following 2AG hydrolysis is directed toward PG biosynthesis. The possibility that endocannabinoid metabolism and subsequent COX-mediated oxygenation of arachidonic acid is perturbed following exposure of macrophages to OP insecticides suggests a novel role for these xenobiotics in vascular wall dysfunction. [Supported by NIH R15ES015348]

PS 2087 ETHYLENE BISDITHOCARBAMATE PESTICIDES MANEB AND MANCOZEB CAUSE TOXICITY IN NORMAL AND TRANSFORMED COLON CELLS.

H. M. Lisa and D. Hardej. *Department of Pharmaceutical Sciences, St. John's University, Queens, NY.*

Ethylene bisdithiocarbamate pesticides maneb (MB) and mancozeb (MZ) are broad range contact fungicides used for eradication of fungal infection on a wide variety of crops. Although reported to possess low acute toxicity to humans, scientific evidence suggests that toxicity does occur. The purpose of this study was to investigate toxicity of MB and MZ in human CCD-Co18, Caco2, and HT29 colon cells. Previous testing in our laboratory has established the acute toxicity of these agents in HT29 and Caco2 cell lines. Significant decreases in cell viability were observed in HT29 cells from 200-400μM and in Caco2 cells from 100-400μM for both compounds. Preliminary data with normal colon cells, CCD-Co18, shows a similar pattern of acute toxicity, with significant decreases in cell viability ranging from 100-400μM for both agents. To investigate chronic toxicity, cells were exposed to concentrations of both agents ranging from 12.5-200μM for HT29 and 0.625-10μM for Caco2 cells for 10 days and viability assessed by the MTT assay. HT29 cells showed significant decreases in cell viability at all concentrations on days 2 through 10 for both agents. Caco2 cells exposed to MB showed significant decreases in cell viability in concentrations 2.5-10μM on days 4 through 8, from 1.25-10μM on day 6, and 5-10μM on day 10. Upon MZ exposure, Caco2 cells showed significant decreases in cell viability on days 4 and 6 from 1.25-10μM, day 8 from 0.625-10μM, and day 10 from 2.5-10μM. To evaluate if cellular injury resulted in lipid peroxidation, thiobarbituric acid reactive substances (TBARS) assay was performed on concentrations 100-200μM for both agents in HT29 cells and 20-60μM for both agents in Caco2 cells. Lipid peroxidation was not observed in either cell line with either compound at the concentrations tested. We conclude that the acute toxicity seen in transformed colon cells is comparable to normal cells at the concentrations tested and that chronic toxicity in HT29 and Caco2 cells occurs at significantly lower doses.

PS 2088 RETINOIC ACID AND TESTOSTERONE LEVELS IN TESTIS AFTER MOLINATE: *IN VITRO* AND *IN VITRO* STUDIES.

F. G. Zuno, B. S. Winder, D. Holstege and M. G. Miller. *Environmental Toxicology, University of California, Davis, CA.*

Molinate (MOL) is a herbicide and testicular toxicant that is metabolically activated via sulfoxidation. Inhibition of esterase activity is seen after molinate administration and it has been proposed that inhibition of hydrolysis of cholesterol esters with decreased availability of cholesterol for testosterone biosynthesis accounts for the testicular effects. An alternate hypothesis is that inhibition of retinyl ester hydrolysis decreases retinoic acid levels in the testis. MOL is also a known inhibitor of aldehyde dehydrogenase (ALDH) and could inhibit ALDH-catalyzed metabolism of retinaldehyde to retinoic acid. To investigate these possibilities, studies were carried out both *in vitro* and *vivo*. In rat Leydig cell cultures, IC50s for inhibition of nonspecific esterase (NSE) activity by MOL and molinate sulfoxide (MSO) were 5.42 μM and 1.9 nM, respectively. These values were more than two orders of magnitude below that required to inhibit testosterone production (750 uM for MOL and 60 μM for MSO) suggesting that the two effects may be unrelated. In testis S9 fractions, neither MOL (800 uM) nor MSO (800 uM) were potent inhibitors of the conversion of retinaldehyde to retinoic acid. To directly measure the effect of MOL on testis levels of testosterone and retinoids in the whole animal, male Sprague-Dawley rats were administered MOL (200 mg/kg in corn oil, ip). Retinoid measurements were made at 6, 12, 48 and 168 h time points after MOL administration. Testis levels of retinol and retinaldehyde were not significantly affected by MOL. However, retinoic acid levels were decreased in a statistically significant manner at the 12 and 48 h time points. In contrast to modest effects on retinoic acid levels, MOL markedly decreased testis levels of testosterone. Overall, the data suggest that NSE inhibition is not directly related to the decrease in testis testosterone levels seen after MOL. Moreover, MOL decreases testis levels of retinoic acid but the magnitude of this effect is much less than the effect of MOL on testosterone.

PS 2089 CHARACTERIZATION OF CHLORPYRIFOS TOXICITY ON THE PANCREATIC BETA CELL LINE RINM5F.

Z. Yan and D. R. Cool. *Pharmacology/Toxicology, Wright State University, Dayton, OH.* Sponsor: C. Sulentic.

Chlorpyrifos (CPF) is a well-known organophosphate (OP) insecticide that inhibits acetylcholinesterase (AChE). Organophosphates have been shown to induce hyperglycemia in both humans and animals that cannot be explained by AChE inhibition alone. Pancreatic β-cells are responsible for secreting insulin and playing a fundamental role in glucose homeostasis. However, no studies have investigated the direct effect of CPF on pancreatic β-cell viability. The aim of this research was to investigate CPF toxicity on the insulin secreting β-cell line RINm5f (RIN). We determined that cytotoxic concentrations of CPF between 50 μM and 200 μM induced a dose-dependent increase in RIN cell apoptosis using ELISA apoptotic assay and caspase 3/7 activation assay ($p<0.05$). Lower doses, i.e., 5-25 μM did not induce apoptosis. Further, low dose CPF's effects on insulin synthesis and secretion was studied. Our results showed that RIN cells treated with CPF for 4 hr exhibited a dose-dependent decrease in insulin secretion in response to stimulation by 50 mM potassium ($p<0.05$). Insulin secretion stimulated by 25 mM glucose also showed significant decreases upon incubation with various doses of CPF ($p<0.05$). Insulin synthesis involves proteolytic cleavage of proinsulin at two specific sites. The enzymes responsible for promoting proinsulin cleavage, i.e, prohormone convertase (PC) 1/3 and 2 (PC2), contain a serine hydrolase catalytic triad, His-Asp-Ser, similar to AChE. CPF modification of PC enzymes similar to AChE, could potentially interrupt the conversion of proinsulin to insulin and disrupt insulin secretion. The results showed that PC2 enzyme activity was significantly inhibited by CPF at concentrations as low as 10 μM ($p<0.01$). In summary, our results show that CPF not only exerts a direct cytotoxic effect at concentrations above 50 μM, but also significantly impairs insulin secretion at lower doses. Low dose CPF could potentially affect endocrine function in vivo by inhibiting the proinsulin processing enzyme PC2.

PS 2090 EARLY DEVELOPMENTAL EXPOSURE TO THE HERBICIDE ATRAZINE RESULTS IN ENRICHMENT OF GENE EXPRESSION ALTERATIONS ASSOCIATED WITH NEUROENDOCRINE DEVELOPMENT AND CARCINOGENESIS.

G. J. Weber[1], S. S. Lewis[2], S. M. Peterson[1], M. S. Sepúlveda[2] and J. L. Freeman[1]. [1]*Health Sciences, Purdue University, West Lafayette, IN and* [2]*Forestry and Natural Resources, Purdue University, West Lafayette, IN.*

Endocrine disrupting chemicals (EDCs) are exogenous agents that alter endocrine system functions and are associated with a myriad of diseases. Atrazine is an herbicide commonly reported to contaminate drinking water supplies in the United States and is a noted endocrine disruptor. Atrazine's mode of action has been shown to be nonreceptor-mediated thus limiting current knowledge on how exposure to atrazine alters endocrine function. In an effort to elucidate the toxic mechanisms of action, we exposed zebrafish embryos to 0.3, 3, and 30 parts per billion (ppb) atrazine shortly after fertilization for 72 hours. A portion of the larvae were collected at the end of the treatment period and prepared for global gene expression analysis, morphological analysis, or washed and allowed to continue to grow. Gene expression data revealed 120 gene targets that were significantly altered in the 0.3 ppb concentration while the 3 ppb and 30 ppb concentrations had 248 and 252 gene targets altered, respectively. Interestingly, 152 of these gene targets were found in both the 3 ppb and 30 ppb analyses. In addition, gene ontology and molecular network analysis using Ingenuity Pathway Analysis software indicated an enrichment of genes associated with neurological disease and neuroendocrine development in the 0.3 ppb gene set. Moreover, in the two higher atrazine concentrations (3 ppb and 30 ppb) the altered gene expression profiles were enriched with several gene targets associated with cell proliferation and cancer development. Future work is now aimed at confirming a panel of cancer-related gene targets using quantitative PCR and assessing lifespan consequences of the early developmental atrazine exposure.

PS 2091 CHANGES IN EXPRESSION OF CRITICAL PROSTATE CANCER GENES UPON EXPOSURE TO ENVIRONMENTAL ANTI-ANDROGENIC PESTICIDE VINCLOZOLIN.

S. Prasad[1], M. Sienkiewicz[1], B. Nguyen[1], S. Ramoju[1], P. O'Reilly[1] and J. Gomes[2, 3]. [1]*Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada,* [2]*Health Science Program, University of Ottawa, Ottawa, ON, Canada and* [3]*Institute of Population Health, University of Ottawa, Ottawa, ON, Canada.*

Prostate cancer (PCa), a highly prevalent cancer in Western countries, is a hormonal disease highly dependent on proper functioning and expression of several regulatory genes. GSTP1, gene involved in detoxification, and NKX3.1, putative tumour suppressor gene involved in regulating normal prostate epithelial growth, are two of the most common downregulated genes in PCa. Vinclozolin (VCZ), an antiandrogenic fungicide used on food crops such as soft fruits and vegetables, acts as an endocrine disrupting chemical (EDC) and its metabolite have a high affinity for the androgen receptors of prostate cells. VCZ has shown to alter the expression of androgen dependant genes and produce transgenerational epigenetic alterations in rat offsprings including atrophy of the prostate. We examined the potential of VCZ to act on the expression of the aforementioned genes, GSTP1 and NKX3.1, in prostate cells. Briefly, androgen-sensitive human PCa cells, LNCaP, and normal human prostate cells, RWPE-1, were exposed to endogenous hormone dihydrotestostorone, and VCZ in a time-dose dependant manner. Treated and control cells were harvested at 12, 24, 48, 72 and 96h after exposure. Changes in gene expression levels were quantified using qRT PCR. Alterations at the protein level was examined by SDS-polyacrylamide gel electrophoresis with subsequent Western blotting. A 1.3-2.2 fold increase in GSTP1 and NKX3.1 mRNA expression over a range of 200uM-5nM VCZ concentrations was observed. These results seem to correlate well with previous in vivo studies showing similar fold changes. Exposures at higher concentrations tend to show reduced GSTP1 and NKX3.1 expression – a possible result of DNA hypermethylation. It is evident that EDCs have the potential to mimic endogenous hormones and alter expression of critical genes yet more work is needed to elucidate the pathway and mechanistic role of EDCs in PCa.

PS 2092 PESTICIDE MIXTURES IN REPTILIAN AND AQUATIC MODELS.

M. H. Feld and I. P. Callard. *Department of Biology, Boston University, Boston, MA.*

Human and wildlife health may be threatened by exposure to a myriad of environmental pollutants. Turtles are an excellent sentinel species due to their long life and wide range of habitation. The pesticides dieldrin and p,p'-DDE were detected in

egg yolk collected from turtles inhabiting a pond impacted by pollutants from the Massachusetts Military Reservation (MMR) Superfund site (Cape Cod, MA). Reproductive abnormalities have been seen in turtles from this site such as altered responses to 17 β-estradiol and gonadotropin signaling, reduced testicular weight, lower epididymal sperm counts, and increased germ cell apoptosis. Exposure to these pesticides in laboratory raised turtles resulted in increased mortality and incidence of deformations. We have also used the zebrafish embryo model to assess the effects of each pesticide, alone and in mixture, and to predict effects in the turtle model. Dieldrin has increased toxicity in the zebrafish model, relative to p,p'-DDE, and microarray analysis of gene expression demonstrated that dieldrin has a much greater effect than p,p'-DDE. A small proportion of genes were found to be induced additively by the pesticides in the mixture, such as Cytochrome (Cyp) P450 24a1like and v-fos oncoprotein. Expression of (Cyp) P450 1a1, a marker of aryl hydrocarbon signaling, was slightly induced following exposure. Cyp 1a1 expression was not found to be altered additively by treatment with these pesticides. We conclude that dieldrin and p,p'DDE may act additively in mixture and changes in gene expression may be correlated with reproductive deficits seen in turtles collected from the MMR Superfund site.

PS 2093 JOINT TOXICITY OF PERMETHRIN AND ATRAZINE TO ZEBRAFISH (*DANIO RERIO*).

P. Wages, A. Trimble and M. Posner. *Biology/Toxicology, Ashland University, Ashland, OH.* Sponsor: P. Wages.

Determining the effects of individual pesticides to non-target organisms in the environment remains an important aspect of toxicological research. However, the characterization of a single pesticide is typically unrealistic in terms of environmental exposure in aquatic systems. In both agricultural and urban landscapes farmers, homeowners, and commercial pesticide applicators are often attempting to control multiple pests through the joint application of herbicides and insecticides. Frequently, atrazine (an herbicide) and permethrin (an insecticide) are applied jointly since each is effective at controlling different pests. This study attempts to elucidate the toxic effect of atrazine and permethrin as a mixture to non-target aquatic species by first using binary mixtures and then examining possible physiological effects using mixtures of specified concentrations. For the first objective, 96-hour static water-only binary mixture toxicity tests were conducted using zebrafish embryos (Danio rerio) as the test organism. Analysis of the predicative models concentration addition (CA) and independent action (IA) revealed that the EC50 to be lower in the CA model, while the IA model provided better predictions of overall mixture toxicity. The second objective's conditions were identical to the previous experiment, but instead of binary mixtures five known concentrations of permethrin (6, 10, 14, 19, and 25 ppb) at three different atrazine conditions (0, 3, and 40 ppb) were conducted. Total body length was assessed after 96-hours revealing that increased concentrations of permethrin resulted in reduced growth with a decrease of 16% total body length by the mixture of 25 ppb permethrin and 3 ppb atrazine. However, a trend of opposition to this growth inhibition occurred with the presence of atrazine. Together, the data suggest that permethrin has the potential to cause both acute and chronic toxic effects to non-target fish species while atrazine could possibly resist permethrin's toxic effect slightly, which could be valuable information for urban and agricultural pesticide applicators.

PS 2094 2, 4-DICHLOROPHENOXYACETIC ACID (2, 4-D): EVALUATION OF ENDOCRINE MODULATING POTENTIAL.

B. Neal[1], M. S. Marty[2], K. Coady[2] and J. C. Lamb[1]. [1]*Center for Toxicology and Mechanistic Biology, Exponent, Alexandria, VA and* [2]*Toxicology & Environmental Research and Consulting, Dow Chemical Company, Midland, MI.*

We have performed a comprehensive weight-of-the-evidence review of both published and unpublished studies of 2,4-D. 2,4-D has a robust mammalian data base, including developmental, sub-chronic and chronic toxicity studies, as well as an F1-extended one generation reproductive toxicity study assessing potential endocrine toxicity, developmental neurotoxicity and immunotoxicity. These data show 2,4-D has a low potential for endocrine modulation (androgenic, anti-androgenic, estrogenic, anti-estrogenic or thyroid active) unless the threshold of renal clearance is exceeded, at doses not relevant to human exposure. In vitro mechanistic and screening assays also support a low likelihood of endocrine activity. The data base for endocrine evaluation of 2,4-D in non-mammalian species is limited; this has recently been supplemented by screening studies in Xenopus laevis tadpoles and fathead minnows; no endocrine-specific effects were observed. In mammals, doses of 2,4-D well above saturation of renal clearance are associated with thyroid findings. These effects are related to displacement of circulating thyroid hormone from transthyretin binding protein, thus enhancing metabolism and excretion of thyroid hormone. A detailed evaluation of thyroid function across life stages in rats, showed only adaptive changes in dams exposed to 2,4-D at a dose exceeding the renal clearance threshold; there were no adverse effects on offspring. Humans have higher levels of a high-affinity thyroid binding protein, i.e., thyroxine binding globulin, compared to the rat, which likely makes humans less sensitive to displacement of thyroid hormone by 2,4-D. The progression and rate of frog metamorphosis, which depends on thyroid function, was examined in 2,4-D-exposed tadpoles, and no treatment-related effects were observed up to the limit concentration. Thus, based on toxicity data in multiple species, there is low potential for 2,4-D to alter endocrine function at environmentally relevant concentrations.

PS 2095 LATE FETAL RESORPTIONS IN RATS INDUCED BY EPOXICONAZOLE OCCUR IN THE PRESENCE OF SIGNIFICANT MATERNAL TOXICITY AND CAN BE ANTAGONIZED BY ESTRADIOL CO-TREATMENT.

S. Stinchcombe[1], S. Schneider[2], I. Fegert[1], V. Strauss[2], S. Gröters[2], M. C. Rey Moreno[2] and B. van Ravenzwaay[2]. [1]*Regulatory Toxicology Pesticides, BASF SE, Ludwigshafen, Germany and* [2]*Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany.* Sponsor: R. Parod.

In a recently published non-GLP study with different azoles, an increase in late fetal resorptions was reported following treatment of rat dams with epoxiconazole (50 mg/kg bw/day) from gestation day (GD) 7-21 (Taxvig et al. 2008). Mechanistic investigations in pregnant Wistar rats with epoxiconazole (CAS No. 133855-98-8) treated by oral gavage from GD 7-18 or GD 7-21 have now revealed that late resorptions at this dose level occurred in the presence of significant maternal toxicity (e.g. reduced corrected body weight gain, signs of anemia). Beside changes in these standard parameters, the increase in late fetal resorptions was associated with a drastically reduced estradiol concentration in maternal serum. By co-administration of dams with estradiol cyclopentylpropionate (0.5 or 1 μg/rat/day by s.c. injection), the increase of late fetal resorptions could be reversed to background control levels.

PS 2096 MICROARRAY ANALYSIS OF GENE EXPRESSION CHANGES IN HUMAN HEPATOCYTES AFTER CHLORPYRIFOS EXPOSURE.

A. D. Wallace[1], R. Shah[2], K. Choi[1], H. Joo[1] and E. Hodgson[1]. [1]*Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC and* [2]*Sciome LLC, Research Triangle Park, NC .*

The organophophorothioate insecticide chlorpyrifos (CPS) is a registered pesticide that is widely used in agriculture on crops such as corn, tree nuts, fruit trees, and soybeans. CPS is bioactivated to the toxic metabolite chlorpyrifos-oxon (CPO) by cytochromes P450 (CYPs), which acts as an insecticide by inhibiting acetylcholinesterase (AChE) activity. Inhibition of AchE results in accumulation of the neurotransmitter acetylcholine in neural junctions resulting in cholinergic toxicity. Human epidemiological studies have led to concerns that human diseases are linked to occupational CPS exposure and in vivo research suggests developmental delays resulting from CPS exposure. Human metabolism of CPS by liver CYPs results in the formation of CPO by a desulfuration reaction that can release a free sulfur that can act to inhibit CYP enzymes, or alternatively, CPS can be metabolized to the non-toxic metabolite 3,5,6-trichloro-2-pyridinol (TCP). Our recent studies have found wide variation in human liver metabolism of CPS and the levels of production of CPO and TCP. To further investigate individual differences in human responses due to CPS exposure, human hepatocytes from multiple donors were treated with CPS and samples were collected at time points of 12 and 24 hours. RNA was prepared and microarray analysis was done using Affymetrix GeneChip Human Genome Arrays to identify genes commonly regulated in multiple individuals, but also to identify unique subsets of genes regulated by different individuals. A number of genes were differentially expressed in response to CPS treatment in multiple individuals, including detoxication enzymes such as CYP2B6, 3A4, and 1A1. Differential expression identified by microarray analysis was verified by quantitative real time PCR. Our findings suggest that exposure to CPS leads to gene expression changes that are largely common across individuals, but also indicate unique attributes.

PS 2097 HUMAN HEPATIC CYTOCHROME P450-SPECIFIC METABOLISM OF CHLORPYRIFOS, PARATHION, METHYL PARATHION, AND DIAZINON.

Y. Tian, C. A. Ellison, R. J. Foxenberg, B. P. McGarrigle, J. B. Knaak and J. R. Olson. *Pharmacology and Toxicology, University at Buffalo, Buffalo, NY.*

Organophosphorous pesticides (OPs) remain a potential concern to human health because of their continuing worldwide use. OPs are metabolized by cytochrome P450s (CYPs) via a dearylation reaction which results in detoxification of the OP or

by a desulfuration reaction which results in a biologically active oxon metabolite that is capable of inhibiting cholinesterase activity. The balance between the rates of desulfuration (activation) and dearylation (detoxification) should, in-part, determine the risk to humans for a given OP. This study investigated the rate of desulfation and dearylation of parathion, methyl parathion, chlorpyrifos, and diazinon. Pooled human liver microsomes were used to asses the metabolism of parathion, methyl parathion, chlorpyrifos and diazinon by measuring the rate of formation of their metabolites, paraoxon and p-nitrophenol (PNP), methyl-paraoxon and PNP, chlorpyrifos-oxon and 3,4,5-trichloropyrindinol (TCP), or diazinon-oxon and 6-methyl-4-pyrmidinol (IMP), respectively. Kinetic parameters (Km & Vmax) for the OPs varied as did the ratio of dearylation/desulfation products. Km and Vmax values for the formation of the active oxon moiety in pooled human liver microsomes were determined to be 17.7μM, 790 pmol/min/mg protein for parathion, 66.8μM, 1844 pmol/min/mg protein for methyl parathion, 2.5μM, 346 pmol/min/mg protein for chlorpyrifos, and 24.5μM, 701 pmol/min/mg protein for diazinon. While, Km and Vmax values for formation of each OPs' detoxified metabolite were 25.1μM, 588 pmol/min/mg protein for parathion, 2.0μM, 123 pmol/min/mg protein for methyl parathion and 16.2μM, 453 pmol/min/mg protein for chlorpyrifos, and 27.5μM, 1277 pmol/min/mg protein for diazinon. Using recombinant human CYPs, CYP2B6, 1A2, and 2C19 were identified as being the primary CYPs involved in OP metabolism. The OP- and CYP-specific kinetic values will be helpful for future use in refining human physiologically based pharmacokinetic/pharmacodynamic models of OP exposure. (EPA STAR grant R833454)

PS 2098 STUDY OF THE INHIBITION OF RECOMBINANT HUMAN CARBOXYLESTERASE 1 AND 2 BY CHLORPYRIFOS OXON, PARAOXON, AND METHYL PARAOXON.

J. A. Crow[1], V. Bittles[1], K. Herring[1], A. Borazjani[1], P. M. Potter[2] and M. K. Ross[1]. *[1]Mississippi State University, Mississippi State, MS and [2]St. Jude Children's Hospital, Memphis, TN.*

Carboxylesterases (CES, EC 3.1.1.1) are serine hydrolases that metabolize a number of xenobiotic and endobiotic compounds containing ester, amide, and thioester bonds. As a result, they are important in the metabolism of many pharmaceuticals. Carboxylesterase 1 (CES1) and CES2 are the two major isoforms of human CEs and have 48% amino acid sequence identity. CES1 is expressed in many tissues throughout the body, whereas CES2 is expressed predominantly in the small intestine, liver, and kidney. The active site serine residue of both CES1 and CES2 reacts covalently with oxons thereby inhibiting the enzyme. Oxons are the bioactivated metabolites of organophosphothionate insecticides formed by cytochrome P450 monooxygenase-catalyzed desulfuration of the parent compound. Assuming a reversible first step in the reaction of an oxon with either CES1 or CES2 (an approach for esterase analysis first described by Main, 1964), we report here the determination of the phosphorylation rate constant (k_p) and the dissociation constant (K_I), as well as the bimolecular rate constant (k_p/K_I) for the inhibition of CES1 and CES2 with either chlorpyrifos oxon, paraoxon, or methyl paraoxon. Based on bimolecular rate constants, CES1 was inhibited 30- to 100-fold faster by each of the three oxons than was CES2. For both CES1 and CES2, chlorpyrifos oxon was the most efficient inhibitor and methyl paraoxon was the least efficient by approximately two orders of magnitude. In general, the bimolecular rate constants correlated with IC_{50} values for each oxon. We are currently determining whether the rank order of reactivity is due to differences in binding affinity (K_I) or rates of phosphorylation (k_p) between oxon and CES. [Supported by NIH R15ES015348]

PS 2099 GLOBAL TRANSCRIPTIONAL PROFILE OF DICHLORVOS EFFECTS IN *DANIO RERIO.*

T. M. Bui Nguyen[1, 2], N. Hussainzada[1, 2], C. Baer[3], J. Lewis[2] and D. Jackson[2]. *[1]Oak Ridge Institute for Science and Education-U.S. Army Center for Environmental Health Research, Fort Detrick, MD, [2]U.S. Army Center for Environmental Health Research-Fort Detrick, Fort Detrick, MD and [3]Excet, Inc., Fort Detrick, MD.*

Dichlorvos (DDVP) induces neurotoxicity and targets the central nervous system. To obtain a global view of the biological processes perturbed by acute toxicity of DDVP, we analyzed gene expression in whole fish and in the liver, the center of detoxification, using pools of RNA from zebrafish exposed for 24 h to a no-observed-adverse-effect and the 96 h LC20 levels. In the liver, DDVP-modulated genes are involved in molecular pathways associated with the metabolism of xenobiotics and lipids, molecular transport, and mitochondrial functions including the IGF-1 signaling pathway, oxidative stress response, programmed cell death, protein ubiquitination pathway and glycogen metabolism. Decreased hepatocellular vacuolation seen in histopathology analysis further supported alterations in glycogen metabolism. Comparative analysis between the whole organism and liver data revealed affected bioprocesses that are common, e.g. glutathione metabolism and β-oxidation. The impacts of DDVP on the renal and muscular systems in whole organism data are corroborated by histopathological findings. The results provide the first glimpse at biofunctions such as the insulin signaling pathway and their putative involvements in pesticides-induced complications. We also propose a novel, high-throughput, and physiologically relevant approach in using whole organisms to detect responses to DDVP. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals in facilities that are fully accredited by the AAALAC. The research described herein was sponsored by the U.S. AMRMC and Military Operational Medicine Research Program.

PS 2100 ABSORPTION AND LIMITED PHARMACOKINETICS OF INHALED SULFURYL FLUORIDE IN NEW ZEALAND WHITE RABBITS.

D. L. Eisenbrandt[1], M. J. Bartels[2], S. M. Krieger[2], D. A. Markham[2], D. L. Rick[2] and J. A. Hotchkiss[2]. *[1]Dow AgroSciences, LLC, Indianapolis, IN and [2]The Dow Chemical Company, Midland, MI.*

Sulfuryl fluoride (SO_2F_2) is a structural and post-harvest fumigant used to control a wide variety of insect pests. The US EPA currently uses the results from a rabbit 90-day inhalation study for risk assessment based on SO_2F_2-dependent neuropathology in this species. To provide comparative data to investigate the physiologic basis for the neuropathology in rabbits compared to rats, six male rabbits were nose-only exposed to 600 ppm SO_2F_2 for 6 h. Blood and plasma samples obtained during exposure (2, 4, and 6 h) were analyzed for SO_2F_2 (blood) and its hydrolysis products, fluoride (F) and fluorosulfate (FSO_3), in plasma. Three rabbits were sacrificed immediately after exposure to determine F and FSO_3 levels in cerebrum, lung and kidney. Serial blood samples were drawn from the remaining 3 rabbits at 2, 4, 8, 12 and 18 h post-exposure and tissues were collected at 18 h post-exposure to determine F and FSO_3 levels. Blood SO_2F_2 was not detected above the lower limit of quantitation (LLQ; 0.8 nmol/ml) at any time during or immediately following the 6 h exposure. Plasma F and FSO_3 levels indicated that inhaled SO_2F_2 was absorbed and rapidly hydrolyzed to F and FSO_3 in rabbits. Plasma F and FSO_3 levels increased in a linear fashion during the 6 h 600 ppm exposure, suggesting that steady state had not been attained. Following exposure, F and FSO_3 were cleared rapidly from the plasma with an elimination half-life of 2.9 and 2.1 h, respectively. Fluoride was detected in plasma at levels above the LLQ (0.9 nmol/ml) through 18 h post-exposure while the plasma FSO_3 levels were below the LLQ (2.6 nmol/ml) by 18 h. Immediately after exposure, both F and FSO_3 were detected in all tissues analyzed, including the cerebrum. This is in contrast to adult and weanling rats exposed 4 h to 300 ppm SO_2F_2 which had cerebral FSO_3 levels ≤ the LLQ at the end of exposure. Species differences in SO_2F_2 absorption, hydrolysis and tissue distribution may contribute to SO_2F_2-dependent neuropathology in rabbits.

PS 2101 KINETIC INTERACTION OF SOLUBLE ESTERASES OF BRAIN AND PHENYLMETHYLSULPHONYL FLUORIDE (PMSF), AN INHIBITOR PROTECTING AND POTENTIATING THE ORGANOPHOSPHORUS DELAYED NEUROTOXICITY.

E. Vilanova, I. Mangas and J. Estévez. *Instituto de Bioingeniería, Universidad Miguel Hernández, Elche, Alicante, Spain.*

The kinetic analysis of esterase inhibition by acylating compounds (organophosphorus carbamates and sulfonylfluorides) is sometimes unable to yield consistent results by fitting simple inhibition kinetic models to experimental data of complex systems. Kinetic data were obtained for phenylmethylsulfonylfluoride (PMSF) tested at different concentrations incubated for around three hours with soluble fraction of chicken brain. PMSF is a protease and esterase inhibitor causing protection or potentiation ("promotion") of organophosphorus delayed neurotoxicity depending of if it is dosed previously or after an inducer of delayed neurotocity and it is unstable in water solution. A kinetic model equation was deduced assuming a multienzymatic system with three different molecular phenomena occurring simultaneously: (1) Inhibition; (2) spontaneous chemical hydrolysis of the inhibitor; (3) ongoing inhibition (inhibition during the substrate reaction). A three-dimensional fit of the model was applied for analysing the experimental data. The best fitting model is compatible with a resistant component (28%) and two sensitive enzymatic entities (61%and 11%). The corresponding second order rate constants of inhibition ($ki=2.3\times10^3$ and 3.6×10^2 $M^{-1}\times min^{-1}$, respectively) and the chemical hydrolysis constant of PMSF ($kh=0.2285$ min^{-1}) were simultaneously estimated. These parameters were similar to those deduced in fixed time inhibition experiments. The

consistency of results in both experiments was considered an internal validation of the methodology. The results were also consistent with a significant ongoing inhibition. This kinetic data are useful for the identification, characterization and understanding the role of soluble esterases in the molecular mechanisms of protection and potentiation.

PS 2102 IMPACTS OF HYPERSALINE CONDITIONS ON ORGANOPHOSPHATE TOXICITY.

L. A. Maryoung, R. Lavado and D. Schlenk. *Environmental Toxicology, University of California, Riverside, Riverside, CA.*

Acclimation to hypersaline environments has been shown in previous studies to enhance the toxicity of thioether organophosphate pesticides to different fish species. One such study showed that phorate toxicity in coho salmon (*Oncorhynchus kisutch*) increased over thirty fold from freshwater acclimated fish to hypersaline acclimated fish. This study also investigated the *in vitro* biotransformation of phorate in coho salmon at varying salinities. It was found that phorate sulfoxide was the predominate metabolite in gills, liver, and olfactory tissues and the rates of formation diminished in a salinity-dependent manner. Phorate-oxon and phorate-oxon sulfoxide were found to be significantly higher in fish acclimated to hypersaline conditions. Phorate oxon was approximately 140 times more potent as an acetylcholinesterase inhibitor relative to phorate. Phorate sulfoxide was 3 fold higher. Overall, these results show that sulfoxidation of thioether organophosphates is not exclusively a detoxification pathway and that rates of secondary oxidation to more potent cholinesterase inhibitors correlated with enhanced hypersaline-dependent toxicity.

PS 2103 METABOLISM OF CHLORPYRIFOS BY ALLELIC ISOFORMS OF CYP2B6, A KEY BIOACTIVATING ENZYME.

A. L. Crane, B. P. McGarrigle and J. R. Olson. *Pharmacology and Toxicology, University at Buffalo, Buffalo, NY.*

Chlorpyrifos (CPF), one of the most commonly used organophosphorous (OP) pesticides, is bioactivated to the active metabolite, chlorpyrifos oxon (CPF-O). CPF-O is a potent inhibitor of acetylcholinesterase, butylcholinesterase, and carboxylesterase, leading to an array of detrimental health effects, including neurotoxicity. CYP2B6 is the most important human enzyme involved in the bioactivation of CPF to CPF-O (Km 0.81 μM; Vmax 12,540 pmol/min/nmol CYP2B6). The potential impact of genetic variability in CYP2B6 on CPF metabolism was investigated in five polymorphic forms of human CYP2B6 (CYP2B6*4, *5, *6, *7 and *18) by assessing the in vitro metabolism kinetics for the formation of the toxic metabolite, CPF-O, as well as formation of the non-toxic metabolite, trichloro-2-pyridinol (TCPy). Studies were done both with mammalian cell expressed protein and insect-cell expressed 2B6 variants to investigate reproducibility of results in different expression systems. 2B6 variants as well as wild type 2B6 were incubated with CPF (0-70uM) and the production of CPF-O and TCPy were analyzed for each isoform by HPLC analysis. Single donor human liver microsomes of known CYP2B6 genotype were also incubated with CPF and examined for the in vitro production of CPF-O and TCPy. Since CYP2B6 is the key bioactivating enzyme at low level, real world exposures to CPF, polymorphisms in CYP2B6 are likely candidates for biomarkers of susceptibility in CPF exposed populations. Future studies will address the role of these genetic variants in human populations and determine if the variability in the in vitro metabolism translates to variability in biomarkers of exposure and effect observed in individuals with occupational exposure to CPF. (Supported in part by EPA STAR grant R833454 and NIH R01 ES016308)

PS 2104 ALTERATIONS IN THE EXPRESSION OF PYRETHROID PESTICIDE METABOLIZING ENZYMES DURING GESTATION IN MICE.

M. C. Fortin[1], L. M. Aleksunes[2] and J. R. Richardson[1]. [1]*Environmental and Occupational Medicine, Environmental and Occupational Health Sciences Institute, Piscataway, NJ and* [2]*Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.*

Pyrethroid pesticides are one of the most widely used classes of pesticides, with agricultural, commercial and residential uses. Pyrethroids are generally thought to be a relatively safe class of pesticides because of low acute toxicity, which is the result of efficient detoxication by various P450s and carboxylesterases (Ces) enzymes. However, significant levels of pyrethroid metabolites have been found in the urine of pregnant women and parent compounds have been detected in meconium. This observation, coupled with the increased sensitivity of developing animals to pyrethroids, raises concern that in utero exposure to pyrethroids may interfere with the developing nervous system. Here, we sought to determine the effect of pregnancy on the expression of hepatic enzymes involved in the metabolism of pyrethroids. Livers were collected from virgin and pregnant C57BL/6 mice (n=3-4) at gestational days (GD) 7, GD11, GD14, GD17, and postnatal days (PND) 1, PND15 and PND30. Hepatic RNA was extracted for quantification of various P450 and Ces isoforms by QPCR. Cyp3a11 and Cyp2c50 expression decreased by about 50% through gestation and lactation, and did not return to control levels by PND30. Cyp2c54, Cyp2c37 and Cyp1a2 mRNA levels decreased by 50% or more during early and mid gestation and returned to normal levels by GD17. Expression of Cyp2b10 mRNA was reduced by 50-60% at GD7 and 11, but increased to 150-200% of control at all points afterward. Cyp3a13 mRNA increased on GD14 and 17, but rapidly returned to control levels. Ces5 was induced by about 80% at GD7, but decreased throughout pregnancy to 35% of the control levels by PND1. Expression of Ces2 and Ces3 were minimally affected by pregnancy. These data demonstrate significant alterations in key enzymes that detoxify pyrethroid pesticides during pregnancy, which could alter perinatal exposure of developing animals to pyrethroids. Supported by NIH ES015991 and ES005022.

PS 2105 ACUTE INHALATION OF AEROSOLIZED CHLORPYRIFOS BY ADULT SPRAGUE-DAWLEY RATS: ABSORPTION, METABOLISM, AND KINETICS OF CHOLINESTERASE INHIBITION IN RED BLOOD CELLS, PLASMA, BRAIN, AND LUNG.

J. A. Hotchkiss[1], S. M. Krieger[1], D. L. Rick[1], M. J. Bartels[1], F. B. Selman[2] and D. R. Juberg[2]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Dow AgroSciences, LLC, Indianapolis, IN.*

Additional toxicokinetic data based on inhalation of chlorpyrifos (CPF) are needed to refine parameter estimates to include lung metabolism in current PBPK/PD models of CPF exposure/response relationships. This study assessed the absorption and metabolism of inhaled particulate CPF and the kinetics of CPF-induced cholinesterase (ChE) inhibition in two phases. First, male and female rats were nose-only exposed for 6 h to 0, 13.3, or 66.7 mg/m^3 CPF (1.4 μm mass median aerodynamic diameter; MMAD). Blood drawn during and after exposure was analyzed for ChE activity in red blood cells (RBC) and plasma. Concentration- and time-dependent inhibition of both RBC and plasma ChE activity were observed with peak inhibition approximately 2 h after exposure. The magnitude of CPF-induced ChE inhibition in female rats was similar or greater than in males. In the next phase, female rats were exposed 6 h to 0, 3.7, 12.9, 22.1, or 53.5 mg/m^3 CPF (1.8 to 1.9 μm MMAD). Post-mortem tissue samples collected during and after exposure were analyzed for ChE activity (RBC, plasma, brain, lung) and CPF, CPF-oxon and 3,5,6-trichloro-2-pyridinol (TCP) levels (whole blood). Total TCP in urine was used to estimate absorbed CPF dose. Peak blood levels of CPF and CPF-oxon were observed during exposure while peak ChE inhibition occurred 2 h or more after exposure. Inhibition of plasma and lung ChE was statistically identified in all exposure groups while inhibition of RBC ChE activity was statistically identified in only the three highest groups. Inhibition of brain ChE activity was statistically identified only in rats exposed to 53.5 mg/m^3 CPF and was 3.5 to 6-fold less than that measured in RBC, plasma, and lung samples from the same rats. Based on these data the NOEC for RBC ChE inhibition = 3.7 mg CPF/m^3 (calculated dose = 0.5 mg CPF/kg) and the NOEC for brain ChE inhibition = 22.1 mg CPF/m^3 (calculated dose = 2.2 mg CPF/kg).

PS 2106 CONSTRUCTION AND VALIDATION OF A HUMAN PBPK/PD MODEL FOR DERMAL CHLORPYRIFOS EXPOSURE UTILIZING HUMAN BIOMARKER DATA.

C. A. Ellison[1], J. B. Knaak[1], R. McDougall[2], P. J. Lein[3, 4], F. M. Farahat[5], K. Anger[3] and J. R. Olson[1]. [1]*University at Buffalo, Buffalo, NY,* [2]*University of Ontario Institute of Technology, Oshawa, ON, Canada,* [3]*Oregon Health & Science University, Portland, OR,* [4]*University of California Davis School of Veterinary Medicine, Davis, CA and* [5]*Menoufia University, Shibin el Kom, Egypt.*

Chlorpyrifos (CPF), a widely used organophosphorus (OP) pesticide, is metabolized either to an inactive metabolite, trichloro-2-pyridinol (TCPy), or to chlorpyrifos oxon (CPF-oxon), which is a potent cholinesterase (ChE) inhibitor. CPF is primarily activated by cytochrome P450 (CYP) 2B6 and detoxified by CYP2C19, while CPF-oxon is metabolically detoxified to TCPy by paraoxonase 1 (PON1). Urinary TCPy levels have been shown to be a reliable biomarker for CPF exposure while blood ChE activity is an indicator of CPF toxicity. In the present study, Egyptian agriculture workers (n=37) had urinary TCPy (daily) and blood butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) activities (every 7-8 days) measured as biomarkers of CPF exposure and effect. Human PBPK/PD modeling

was conducted utilizing the exposure and effect biomarker data and CYP-specific kinetic rate constants for CPF metabolism. Urinary TCPy levels ranged from 5.3 – 30,107 μg/g creatinine, while BuChE and AChE activity was inhibited up to 100 and 95%, respectively. A statistically significant inverse correlation was observed between urinary TCPy and blood BuChE and AChE activities. The no effect level or inflection point of the exposure-effect relationships had an average urinary TCPy level of 114 μg/g creatinine for BuChE and 3,161 μg/g creatinine for AChE. Our findings are the first to demonstrate a dose-effect relationship between urinary TCPy and both plasma BuChE and RBC AChE in humans exposed occupationally to CPF. Human PBPK/PD modeling simulations for a dermal CPF exposure were able to accurately simulate the human exposure and effect biomarker data. (NIH R01 ES016308 and EPA STAR grant R833454)

PS 2107 INTEGRATION OF TOXICOKINETICS (TK) INTO GUIDELINE TOXICITY STUDIES OF A NEW AGROCHEMICAL.

S. A. Saghir[1], D. L. Rick[1], A. J. Clark[1], J. L. Staley[1], M. J. Bartels[1], C. Terry[2] and R. Billington[2]. [1]*The Dow Chemical Co., Midland, MI and* [2]*Dow AgroSciences, Milton Park, United Kingdom.*

Integrated TK data (without satellite animals) provide information on the rate, extent and duration of systemic exposure across doses, species, strains, sexes and life stages within a toxicology program and may also be able to improve human risk assessments. While routine for pharmaceuticals, TK assessments of non-pharmaceuticals are still relatively rare and have never before been included in a full range of guideline studies for a new agrochemical. In order to better understand the relationship between systemic dose and toxicity of X11422208, TK analyses were implemented in all short and long term studies including reproduction and developmental tests. Consistent across species (mice, rats, rabbits and dogs) was the rapid and complete absorption of X11422208, little to no metabolism, and elimination primarily via urine. Female animals more rapidly eliminated X11422208 from plasma than males. Dose-proportionality of systemic exposure was maintained across studies except for mice, where the 90-day plasma AUC24h was supralinear in males at ≥750 ppm (saturation of elimination) and sublinear in females at ≥1500ppm (saturation of absorption). Therefore, the kinetically-derived maximum doses (KMD) for the mouse 18-month carcinogenicity study were 750 ppm (male) and 1500 ppm (female). In addition to leveraging TK data to guide dose level selection, the integrated program was also used in rabbits to select the most appropriate method of oral administration. Administration of X11422208 by gavage to rabbits resulted in a 3-fold diurnal fluctuation between Cmax and Cmin plasma concentration with a maximum tolerated dose (MTD) at 12 mg/kg/day. Dietary exposure reduced the diurnal fluctuation in plasma concentration without changing the AUC24h and afforded a higher MTD of 32 mg/kg/day. In summary, integrated TK data across toxicity studies, without the use of additional animals, provided data critical to understanding differences in response among species to X11422208. Such data should also be useful in human risk assessment of chemicals.

PS 2108 TOXICOKINETIC - TOXICODYNAMIC RELATIONSHIPS IN CASES OF GLYPHOSATE HERBICIDE EXPOSITION.

A. Anadon, M. A. Martínez, I. Ares, E. Ramos, V. Castellano, M. Martínez and M. R. Martínez-Larrañaga. *Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain.*

Glyphosate (N-phosphonometil-glicine) is an organophosphate herbicide with no anticholinesterase activity. Accidental ingestion of glyphosate is associated with effects such as spasms, panting, shaking, hind-leg ataxia and muscle spasms. Because of toxicological data are needed to define the safety use of this herbicide, this study evaluated neurochemical effects of glyphosate and the value of Toxicokinetic-Toxicodynamic (TK-TD) relationships. The present study correlated the content of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA), and the glyphosate concentrations in brain tissues as well as plasma kinetic parameters. Two experiments were carried out: (1) Male Wistar rats treated with glyphosate (single dose of 400mg/kg, per os) were killed at different time period after treatment and blood and brain samples were collected to determine glyphosate levels by HPLC-UV; (2) male Wistar rats treated with glyphosate (400mg/kg, per os, for 5 days) and male Wistar rats treated with corn oil (control animals) were killed 24h after dosing and brain regions isolated and content of 5-HT and 5-HIAA quantified by HPLC-ED. The results showed that glyphosate was distributed into SNC. Glyphosate levels detected in brain were 4-10μg/ml. Glyphosate was partially and slowly absorbed with the following plasma kinetic parameters: T1/2a=2.29h, Cmax=4.62μg/ml and Tmax=5.16h. The plasma elimination half-life (T1/2β) was 14.38h. Glyphosate decreased 5-HT levels in frontal cortex (28% P<0.001), hypothalamus (25% P<0.01), hippocampus (33% P<0.01) and striatum (38% P<0.001) respect to controls. The data presented herein suggest that an accumulation of glyphosate with time might be closely associated with its toxicity and TK-TD relationships should be considered for the risk assessment of this herbicide. This work was supported by projects Ref. BSCHGR58/08(UCM), Ref.No S2009/AGR-1469(CAM) and Consolider-Ingenio 2010 No.CSD2007-063(MEC), Spain.

PS 2109 PROPICONAZOLE INDUCES ALTERATIONS IN THE HEPATIC METABOLOME OF MICE: RELEVANCE TO PROPICONAZOLE-INDUCED HEPATOCARCINOGENESIS.

S. Nesnow, T. Moore and W. Padgett. *NHEERL/ISTD, U.S. EPA, Research Triangle Park, NC.* Sponsor: S. Hester.

Propiconazole is a mouse hepatotumorigenic fungicide and has been the subject of recent mechanistic investigations on its carcinogenic mechanism of action. The goals of this study were: 1. To identify metabolomic changes induced in the liver of mice by increasing doses of propiconazole; 2. To interpret these results with key previously reported biochemical, transcriptomic and proteomic findings obtained from mouse liver under the same treatment conditions; 3. To relate these alterations to those associated with the carcinogenesis process. Propiconazole was administered to male CD-1 mice in the feed for 4 days with 6 mice per group (500, 1250, and 2500 ppm). Endogenous biochemicals were profiled using LC-MS/MS and GC-MS methods and 261 were detected. The most populous biochemical class detected was lipids, followed by amino acids and then carbohydrates. Nucleotides, cofactors and vitamins, energy, peptides and xenobiotics were also represented. Of the biochemicals detected 159 were significantly altered by at least one dose of propiconazole and may showed strong dose responses. Many alterations in the levels of biochemicals were found in the glycogen metabolism, glycolysis, lipolysis, carnitine and the TCA cycle pathways. Several groups of metabolomic responses were ascribed to the metabolism and clearance of propiconazole: glucuronate, glutathione and cysteine pathways. Groups of metabolic responses supported hypotheses on key events that can lead to propiconazole induced tumorigenesis: oxidative stress and increases in the cholesterol biosynthesis pathway. Groups of metabolomic responses identified biomarkers associated with neoplasia: increases in polyamine and sarcosine biosynthesis, and increases in pseudouridine levels. These results extended the companion transcriptomic and proteomic studies and provided a more complete picture of propiconazole's effects in mouse liver. This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

PS 2110 EXPRESSION OF CYPS 2B1/2B2, BUT NOT CYPS 1A1 OR 1A2, ARE INDUCED IN THE LIVER FROM RATS FED MIXTURES OF THE PESTICIDES DIELDRIN, DICHLORVOS, PERMETHRIN, DICOFOL, AND ENDOSULFAN.

S. Ramos da Silva, C. S. Franchi, J. V. de Camargo and D. Elgui de Oliveira. *Pathology, São Paulo State University (UNESP) Medical School, Botucatu, SP, Brazil.* Sponsor: D. Wolf.

Introduction: Human exposure to pesticide residues that contaminate edible agricultural products is an important public health concern. However, the biological effects of dietary exposure to the mixture of these compounds are largely unknown. This study evaluated the effects of two dietary mixtures of five pesticides on the induction of rat liver CYP450 enzymes. The pesticides dieldrin, dichlorvos, permethrin, dicofol and endosulfan were chosen because their residues were found by the Brazilian Agency for Health Surveillance (ANVISA) in tomatoes regularly available over-the-counter for Brazilian consumers. METHODS: Lewis male rats were exposed through diet to each of the pesticides or to mixtures made up with all of them in different low-dose concentrations. Pesticides were added to feed according to their respective NOEL (MEX1, Non-effective Levels) or LOAEL, LEL or LOEL (MEX2, Effective Levels). Control animals received the unadulterated rodent chow. After 8 weeks animals were euthanized and liver fragments were obtained for molecular analysis. Quantitative reverse-transcription PCR (qRT-PCR) was used to assess the mRNA levels of CYP1A1, 1A2 and CYP2B1/2B2 in liver tissues. Differential expression in treated groups was normalized to the expression of the rat albumin gene, and calculated as fold-change in respect to the mean expression found for control animals. RESULTS: Pesticides or mixtures have increased the transcriptional levels of CYPs 2B1/2B2, but not CYPs 1A1 or 1A2, in the liver of treated animals. Compared to controls, significant differences in the expression of CYPs 2B1/2B2 were observed in the livers of animals fed MEX1 and MEX2 or to the isolated pesticides dicofol (LOEL), dieldrin (LOAEL) and permethrin (LEL). CONCLUSION: Exposure to pesticides alone or in mixtures at relatively low doses changed the expression of the liver CYP 2B1/2B2 mRNA, what may have impact on the metabolism of xenobiotics.

PS 2111 CYTOCHROME P450 3A5 GENOTYPE IS CORRELATED WITH ACETYLCHOLINESTERASE INHIBITION LEVELS AFTER EXPOSURE TO ORGANOPHOSPHATE PESTICIDES.

Z. Guerrette[1], E. G. Moreira[1, 3], W. C. Griffith[1], G. D. Coronado[2], E. M. Vigoren[1], X. Yu[1], B. Thompson[1, 2] and E. M. Faustman[1]. [1]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA,* [2]*Fred Hutchinson Cancer Research Center, Seattle, WA and* [3]*State University of Londrina, Londrina, PR, Brazil.*

Organophosphate pesticides (OPs) can pose occupational health hazards for farmworkers. Genetic variation in the genes responsible for activating OPs to the active oxon form, including cytochrome P450s (CYP450s), can impact the formation of the oxon intermediate and thereby potentially affect cholinesterase inhibition. In 2005, we conducted a longitudinal study involving farmworkers and non-farmworkers in the Yakima Valley, Washington State. Urine, blood, and buccal samples were collected during times of pesticide application and non-spray periods. Urine was analyzed for the non-specific OP metabolites and field test kits were used to measure red blood cell (RBC) acetylcholinesterase (AChE) activity. DNA was isolated from buffy coat preparations and was used for genotyping with the Affymetrix Drug Metabolizing Enzyme and Transporter (DMET) Plus platform. We previously established a positive linear relationship between the OP azinphos methyl (AZ) in blood or urinary dimethylthiophosphate (DMTP) levels and AChE inhibition. We investigated if genotypes for key CYP450 genes might modify this relationship and our analysis has identified genetic variation in the CYP3A5 gene (6986A>G, rs776746) (ANOVA, $p < 0.05$). Post-hoc testing showed that individuals with the G/G genotype had higher AChE inhibition per unit AZ or DMTP compared to A/A individuals. Since the G allele produces a splice defect that is associated with lower enzyme activity, future PBPK modeling will determine if genotypic variation at this locus could be used to identify individuals who may be more sensitive to OP-associated effects. Supported by grants T32 ES007032 and 2-P01-ES009601 from NIEHS and RD-8370901 and STAR RD-83273301 from EPA. Contents are the responsibility of the author and do not necessarily represent views of NIEHS, NIH or EPA.

PS 2112 GLUTATHIONE S-TRANSFERASE POLYMORPHISMS INFLUENCE THE LEVEL OF OXIDATIVE DNA DAMAGE IN SOYBEAN PESTICIDE APPLICATORS.

M. A. Carballo[1], M. Porcel de Peralta[2], E. Kleinsorge[2] and M. Simoniello[1, 2]. [1]*Bioquímica Clínica, Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Buenos Aires, Argentina and* [2]*Bioquimica Clínica, CIGETOX. INFIBIOC. Facultad de Farmacia y Bioquímica. UBA, Buenos Aires, Buenos Aires, Argentina.*
Sponsor: O. Olivero.

Occupational exposure of soybean crops is characterized by alternating periods of intense pesticide spraying and reduced or no activity. Damage Index Comet Assay (DICA), oxidative DNA damage introducing the modification by adding FPG protein (FPG Sites) and Buccal Cells Micronucleous (BCMN) were investigated in 17 pesticide applicators and 17 matched controls. Samples were performed during intensive pesticide treatments. Each donor was genotyped for glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) polymorphic genes involved in xenobiotic metabolism to assess their potential role in individual genotoxic response to pesticide exposure. DICA, FPG Sites and BCMN were increased in exposed workers when compared with controls ($p<0.001$). Significant influence of phenotype composition on genotoxic response was: an increase in DICA levels in the GSTT1 positive individuals compared with the GSTT1 nulls ($p<0.05$) and FPG Sites in the GSTM1 positive individuals in relation to nulls. In addition, a possible interaction was observed between smoking and GSTM1 genotype in the FPG Sites, GSTM1 null smokers, earlier reported to have an elevated risk for lung cancer, showing higher ($p<0.05$) frequencies than GSTM1 positive smokers. The incorporation of phase II polymorphisms metabolizing enzyme glutathione S-transferase would be important as susceptibility biomarkers in workers occupationally exposed to pesticides.

PS 2113 HYDROLYSIS OF ORGANOPHOSPHATE AND MODEL SUBSTRATES IN AFRICAN AMERICAN AND CAUCASIAN SOUTHERNERS BY SERUM PARAOXONASE-1 (PON1) AND RELATIONSHIP TO ATHEROSCLEROSIS.

H. Coombes, A. Crow, M. Dail, H. Chambers, R. Wills, E. Meek and J. Chambers. *Mississippi State University, Mississippi State, MS.*

Paraoxonase-1 (PON1) hydrolyzes organophosphates (OPs) and has antioxidant and cardiovascular system-protective properties. PON1, associated with high density lipoprotein (HDL) particles, protects against atherosclerosis (ATH) by hydrolyzing oxidized low density lipoproteins (LDL). An analysis of enzyme activities along with comparisons to demographic and clinical information was conducted for 200 adults: 80 African Americans (40 male, 40 female) and 120 Caucasians (60 male, 60 female). Functional genotype of Q192R polymorphism was determined from plots of diazoxon (DZO) hydrolysis vs. paraoxon (PO) hydrolysis. Non-OP substrates (phenyl acetate, PhAc, and 4-[chloromethyl] phenyl acetate, CMPA) were also investigated. Population distribution into functional genotypes using plots of rates of hydrolysis of the model substrate pair PhAc vs. CMPA was less clear than those of the OP substrate pair DZO vs. PO. African Americans had higher POase activities but lower DZOase activities than did Caucasians, consistent with African Americans having a lower proportion of the QQ genotype (QQ18%, QR45%, RR37%), than did Caucasians (QQ46%, QR45%, RR 9%). The pattern of chlorpyrifos-oxon (CPO) hydrolysis by direct assay was more similar to that of PO than of DZO in which the RR genotype had higher activity than the QQ genotype. CPO hydrolytic activity was generally correlated with hydrolysis of both OP and model substrates. African Americans had higher CMPA activities but lower PhAc activities than did Caucasians similar to POase and DZOase, respectively. Associations of ATH with clinical factors using odds ratios showed POase activities, measured by CMPA hydrolysis, has protective effects towards ATH ($p<0.05$). Multivariable modeling showed age, gender, smoking history, hypertension, and high LDL-cholesterol to be the strongest risk factors for ATH ($p<0.05$) while POase activities measured by PhAc hydrolysis showed a protective effect. (Supported: NIH R21 ESO15107).

PS 2114 INTRAVENTRICULAR CONDUCTION DELAY AFTER GLYPHOSATE-SURFACTANT HERBICIDES INTOXICATION.

Y. Tseng[1], C. Chen[1, 2], C. Huang[4], W. Chen[1] and D. Hung[1, 2, 3]. [1]*Department of Emergency, China Medical University Hospital, Taichung, Taiwan,* [2]*Toxicology Center, China Medical University Hospital, Taichung, Taiwan,* [3]*Graduate Institute of Drug Safety, College of Pharmacy, China Medical University, Taichung, Taiwan and* [4]*School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan.*

Glyphosate-surfactant herbicides (GSH) are used in agriculture world-widely. The commonly used GSH contains 41% isopropylamine salt, water, and a variable amount of surfactant. The main surfactant is polyoxyethyleneamine (POEA). The major causes of death following GSH intoxication remain unclear. It has been suggested that respiratory failure, pulmonary edema, metabolic acidosis, hyperkalemia may predict the risk of poor outcome. Experimental studies show that surfactant is mainly attributed to these toxicities. Lin et al reported a young GSH-intoxicated patient with cardiogenic shock and arrhythmia. Vasorelexation and inhibition of heart twitch tension were observed in the study by Chan et al. Ventricular arrhythmia often developed and are fatal in humans. Here we reported 3 cases of GSH intoxication presented with widening of the QRS complex on the electrocardiogram (EKG) initially. Then they developed unconsciousness, respiratory failure, metabolic acidosis and shock during admission. After aggressive supportive care and hemodialysis, the QRS complex gradually narrowed, sinus rhythm returned, and the hemodynamic status improved. The exact mechanism of intraventricular conduction delay is unknown. The cardiovascular effect of GSH can be complicated. Since shock is the major cause of death and usually develops early after GSH exposure. We though EKG is rapid and useful as both a screening tool for GSH exposure and as a prognostic indicator.

PS 2115 GENETIC DAMAGE AND GENE EXPRESSION IN PESTICIDE RETAILERS FROM NAYARIT, MEXICO.

E. Rojas[1], S. Lara-Montoya[1], P. Ostrosky[2], M. Sordo[2], M. Medina-Díaz[1], L. Robledo-Marenco[1], A. Benitez-Trinidad[1], B. Quintanilla-Vega[3] and M. Bermúdez de León[4]. [1]*Laboratorio de Contaminación y Toxicología Ambiental, Universidad Autónoma de Nayarit, Tepic, Nayarit, Mexico,* [2]*Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico, D.F., D.F., Mexico,* [3]*Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del IPN, Mexico, D.F., D.F., Mexico and* [4]*Laboratorio de Biología Molecular, Centro de Investigación Biomédica del Noreste-IMSS, Monterrey, Nuevo León, Mexico.*

The widespread use of pesticides represents a risk to the environment and to the health of human populations. Several studies have reported genetic damage in individuals exposed to pesticides, and a high frequency of genetic damage has been associated with the development of cancer. In this study, the cytokinesis-block micronucleus (CBMN) assay was used to measure micronuclei frequency (MNi), nuclear buds (NBUDs), nucleoplasmic bridges (NPBs), necrotic cells, apoptotic cells, and the nuclear division index in lymphocytes from pesticide retailers ($n = 20$) and control non-exposed individuals ($n = 20$) from Nayarit, Mexico. Gene expression was examined in this population using microarrays in a ScanArray 4000 BioPackard system with the software BRB-ArrayTools. The mean MNi was 2.15 (range

0-9) in the pesticide retailers compared to 2.75 (range 0-8) in the control group (p=0.18). There were no statistically significant differences in the frequency of NPBs, BUDs, necrotic cells, or apoptotic cells between the pesticide retailers and the control group. However, lymphocytes from pesticide retailers displayed a higher nuclear division index than those from controls (p <0.05). The results of the microarray assays indicate that pesticide retailers had differences in the expression of some genes involved in metabolic pathways, cancer pathways, cytokine-cytokine receptor interactions, gap junctions, TGF-beta signaling pathways, ubiquitin mediated proteolysis, and the cell cycle, compare to the control group. These results suggest that pesticide exposure may affect cell proliferation kinetics and gene expression. This study was supported by CONACyT-Mexico, Grant C01-4416.

PS 2116 ACETYLCHOLINESTERASE ACTIVITY AND GENE EXPRESSION IN URBAN SPRAYERS FROM NAYARIT, MEXICO.

M. Fuentes-Reyes[1], E. Rojas[1], P. Ostrosky[2], M. Sordo[2], M. Medina-Díaz[1], L. Robledo-Marenco[1], S. Lara-Montoya[1], A. Benitez-Trinidad[1], B. Quintanilla-Vega[3], M. Bermúdez de León[4], B. Barrón-Vivanco[1, 3], I. Girón-Pérez[1], C. González-Arias[1] and F. Herrera-Moreno[1]. *[1]Laboratorio de Contaminación y Toxicología Ambiental, Universidad Autónoma de Nayarit, Tepic, Nayarit, Mexico, [2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, D.F., Mexico, D.F, Mexico, [3]Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del IPN, D.F., Mexico, D.F., Mexico and [4]Laboratorio de Biología Molecular, Centro de Investigación Biomédica del Noreste-IMSS, Monterrey, Nuevo León, Mexico.*

Pesticides are used worldwide and exposure to these compounds has been associated with several adverse effects. The aim of this study was to examine the pattern of pesticide use, acetylcholinesterase (AChE) activity, and gene expression in urban pesticide sprayers and a control group from Nayarit State, Mexico. In this cross-sectional study, the population consisted of 20 male urban sprayers and 20 controls (both groups aged 18-55 years). A structured questionnaire was used to obtain information about the subjects' patterns of pesticide use, anthropometrical characteristics, lifestyle and medical history. AChE activity was determined spectrophotometrically and gene expression was analyzed using microarrays in a ScanArray 4000 Bio-Packard system. Results indicate that pesticides used to fumigate the urban environments (houses, hospitals, schools and others) are pyrethroids (40.6%), followed by coumarins (15.7%) and organophosphates (15.6%). The geometric mean of AChE activity was 32.3 U/g Hb (range 24.4 -38.9 U/g Hb) in urban sprayers and 33.9 U/g Hb (range 24.5-45.3 U/g Hb) in the control group (p=0.33). Preliminary results of gene expression analysis suggest that pesticide sprayers display differences (up and down) in the expression of some genes involved in the regulation of cellular processes including cell division, differentiation, motility, adhesion and death, compared to the control group. In conclusion, these results suggest that pesticide exposure in urban sprayers may modulate gene expression. This study was supported by Conacyt-FOMIX-Mexico Grant 2008-CO2-92435.

PS 2117 ESTIMATING EGYPTIAN COTTON FIELD WORKER EXPOSURE TO THE ORGANOPHOSPHORUS PESTICIDE PROFENOFOS.

O. A. Dadson[1], C. A. Ellison[1], S. T. Singleton[1], L. Chi[1], F. M. Farahat[2] and J. R. Olson[1]. *[1]University at Buffalo, Buffalo, NY and [2]Menoufia University, Shibin el Kom, Egypt.*

Profenofos is a thiophosphate organophosphorus (OP) pesticide which is used on a variety of agricultural crops. Unlike the more commonly used phosphorothioate OP pesticides, thiophosphate OP pesticides do not require metabolic activation to inhibit B-esterases such as acetylcholinesterase, butyrylcholinesterase and carboxylesterase. Metabolic detoxification of profenofos results in the inactive metabolite 4-bromo-2-chlorophenol (BCP) which is readily excreted in urine and can be used as a reliable biomarker of profenofos exposure. In the present study, Egyptian agriculture workers (n=37) with job titles of pesticide applicator, technician or engineer, were studied during 8 to 10 consecutive days of profenofos application to cotton fields. Urinary BCP concentrations were measured daily as a biomarker of profenofos exposure. Urinary BCP concentrations, ranging from 0.9 - 8,053 μg/g creatinine, varied among the three different job categories, with the applicators being consistently higher than technicians and engineers. With the assumption that all BCP excreted in urine is derived from a profenofos exposure, estimates of daily absorbed profenofos doses were made. The estimated absorbed profenofos dose for individuals ranged from 0.037 - 327 μg/kg/day. This is one of the first reports to quantify the magnitude of urinary BCP levels and internal profenofos dose in humans following an occupational exposure to profenofos. Future studies will identify the relationship between urinary BCP levels and neurobehavioral deficits and attempt to identify novel biomarkers, including genetic markers of susceptibility. (NIH R01 ES016308)

PS 2118 THE CONTRIBUTION OF HANDS TO THE DERMAL PESTICIDE EXPOSURE OF STRAWBERRY HARVESTERS.

G. Sankaran[1, 2], L. Chen[2], Z. Chen[1, 2], T. Lopez[1, 2], H. Vega[2] and R. I. Krieger[1, 2]. *[1]Environmental Toxicology Graduate Program, University of California, Riverside, CA and [2]Personal Chemical Exposure Program, Department of Entomology, University of California, Riverside, CA.*

When harvesters use light rubber latex gloves; the gloves may reduce pesticide exposure, cosmetically protect the skin from accumulation of dirt and juice, and be useful as a food safety measure. Potential harvester pesticide exposure (μg/d) may be expressed as dislodgeable foliar residues (DFR, μg/cm2) x empirical harvester transfer coefficient (cm2/h) x h/d. DFR is important when workers contact treated foliage since part is transferrable and accumulates to gloves. Hands contribute 50%-90% of total strawberry harvester pesticide exposure. Harvesters wear gloves as required PPE at DB Specialty Farms, Santa Maria, CA. Pesticide Glove Residues (PGR) are the part of DFR accumulated during harvesting. Malathion (M) and fenpropathrin (F) insecticides were sprayed (lb/A). DFR at 5, 8, 12 and 15 d for M ranged from 0.006 to 0.38 μg/cm2 (Pearson's Correlation coefficient R=-0.78, Alpha p-value=0.00) and from 0.012 to 0.03 μg/cm2 (R= -0.81, p-value=0.00) for F. M residues on randomly collected gloves after a 2 to 2.5 h work ranged 15 to 900 ug/pair (R= -0.86, p-value=0.00) and F residues ranged 120 to 1300 ug/pair(R= -0.65, p-value=0.00). Both total leaf surface residues and transferable accumulated glove residues follow an exponential decay pattern reaching steady low levels after 2 weeks which is highly significant (p-value < 0.01). The DFR dissipation t1/2 for M and F were 1.6 and 2.8 d respectively. The corresponding PGR t1/2s were 2.0 and 4.3 d. PGR is a dynamic measurement of transferable DFR. A glove transfer coefficient is the amount of residue transferred from foliage to gloves during harvesting in unit transfer area/unit time period. Glove transfer coefficient relates PGR to transferable or available DFR. A dermal-based exposure model using non-invasive passive dosimetry is particularly attractive for harvester exposure assessment. Partial support from an SOT/DTSS Battelle Student Research Award is gratefully acknowledged.

PS 2119 PRELIMINARY RESEARCH FOR A MONITORING PROGRAM FOR PESTICIDE RESIDUES ON MARKET VEGETABLES IN MEXICO.

M. L. Aldana-Madrid[1], M. I. Silveira-Gramont[1], A. I. Valenzuela-Quintanar[3], G. Rodríguez-Olibarría[1], P. Grajeda-Cota[3], F. G. Zuno[2] and M. G. Miller[2]. *[1]Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Hermosillo, Sonora, Mexico, [2]Environmental Toxicology, University of California, Davis, CA and [3]Centro de Investigación en Alimentación y Desarrollo, Hermosillo, Sonora, Mexico.*

Systematic monitoring of pesticide residues in fresh vegetables is not implemented in Mexico. There is minimal information about grower use patterns and time to harvest of treated crops. In the absence of data, it is largely unknown what level of pesticide contamination is present in Mexican food crops. The present research was conducted to evaluate the presence of pyrethroid insecticide residues in tomato (Solanum lycopersicum), lettuce (Lactuca sativa), green pepper (Capsicum annuum), white onion (Allium cepa), green onion (Allium schoenoprasum), potato (Solanum tuberosum) and saladette tomato (Lycopersicum esculentum) produced and/or consumed in Sonora, Mexico. The pyrethroids studied were cyhalothrin, cyfluthrin, cypermethrin, fenvalerate, and deltamethrin. Cluster sampling was carried out in 2008 during March-April, and October-November in the markets and fields of several rural development districts. Pyrethroids were extracted and quantified by matrix solid-phase dispersion technique and gas chromatography with electron capture detector. It was found that 9 % of 345 samples tested positive; these samples ranged from 0.004 to 0.573 mg/kg of pyrethroid residues. One green onion sample with cypermethrin, and two potato samples with cyfluthrin exceed the limits allowed by the Codex Alimentarius, and the Mexican Official Norms. The cause of these illegal residues was mainly due to a recent application in the field.

PS 2120 IMPLICATIONS OF ORGANOPHOSPHATE (OP) PESTICIDES IN FOOD GRAIN.

R. Dufault[2] and S. Gilbert[1]. *[1]INND, Seattle, WA and [2]Food Ingredient and Health Research Institute (FIHRI), Honolulu, HI.*

Organophosphate pesticides continue to be widely used by the agricultural industry in the United States. The Centers for Disease Control tracks exposure to these chemicals or their metabolites through the National Biomonitoring Program. A review of the pesticide residue data gathered by the USDA Pesticide Data Program was conducted during this study to determine which foods frequently contain

residues of organophosphates or their metabolites. Between 2004 and 2008, fruit, vegetable, nuts and grains were routinely collected and analyzed for a variety of pesticide residues. Per capita availability data was also obtained from the USDA to determine how much Americans consume of each commodity. The results of this review indicate that wheat and corn are the commodities most likely contributing to organophosphate exposure in children. Percentage of wheat samples with detectable concentrations of chlorpyrifos were reported as 20.8, 23.1, and 16.7 during 2004, 2005, and 2006, respectively. Similarly, percentage of wheat samples with detectable concentrations of malathion were reported as 49.4, 66.9, and 63.0 during 2004, 2005, and 2006, respectively. Estimated per capita wheat consumption was and still is presumably approximately 95 pounds per year. Percentage of corn samples with detectable concentrations of chlorpyrifos were reported as 30.0 and 17.8 during 2007 and 2008, respectively. Percentage of corn samples with detectable concentrations of malathion were reported as 37.9 and 33.7 during 2007 and 2008, respectively. Estimated per capita corn consumption decreased from 52 to 49 pounds from 2007 to 2008. Corn is widely used for the production of sweeteners such as high fructose corn syrup. Children are at risk of exposure to multiple pesticide residues from consuming the very same commodity and cumulative exposures are likely to occur. More research needs to be conducted to determine the organophosphate residue content found in food products made from grain. Exposure to multiple organophosphate pesticides has been linked to the development of learning disabilities in children.

PS 2121 HIGH-THROUGHPUT ASSESSMENT OF CELLULAR MANGANESE (MN) IDENTIFIES NOVEL MECHANISM UNDERLYING A MN DEFICIT OF HUNTINGTON'S DISEASE MODEL.

G. Kwakye[1], D. Li[1], O. Kabobel[1], M. Wegrzynowicz[1], K. M. Erikson[2], M. Aschner[1] and A. B. Bowman[1]. [1]*Neuroscience, Vanderbilt University Medical Center, Nashville, TN and* [2]*Nutrition, University of North Carolina, Greensboro, NC.*

Expression of mutant Huntingtin (Htt) is associated with impaired manganese (Mn) accumulation in a striatal cell line and mouse models of Huntington's disease (HD). To evaluate this mechanism, we have developed and validated a high-throughput fluorescence-quenching based assay (Cellular Fura-2 Manganese Extraction Assay - CFMEA) to measure Mn transport kinetics and storage properties in cultured striatal cells and rodent tissues. We have applied CFMEA to confirm the impaired Mn uptake observed in mutant striatal cells following 27 hours Mn exposure. Here, we demonstrate that the impaired Mn phenotype in mutant striatal cells is observed as early as 1 hour following 200 μM Mn exposure. In addition, this deficit is not dependent on other di-/trivalent metals, serum proteins, or known co-transporter ions in the culture media and exposure buffers. Moreover, mutant striatal cells' Mn uptake deficit is independent of the divalent metal transporter 1 (DMT1) and 2-APB blockable channels. Importantly, mitochondrial function has been reported to be impaired in mutant striatal cells. Thus, we hypothesized that expression of mutant Htt would impair the mitochondrial regulatory functions of metal ion homeostasis via the PPARγ mediated pathway. To test this hypothesis, we utilized a PPARγ agonist and antagonist and failed to observe an influence of the PPARγ mediated pathway on the Mn deficit in mutant striatal cells. In spite of similar instantaneous Mn uptake and efflux rates between wildtype and mutant striatal cells, we demonstrate that there is a significant decrease in net Mn storage capacity in mutant compared to wildtype striatal cells. Finally, we have applied CFMEA in mouse brain tissues to assess temporal and spatial Mn accumulation. In conclusion, our data show that the Mn deficit in mutant striatal cell lines is likely due to impairment of Mn storage capabilities and not uptake. Support: ES016931, ES016931-02S1, ES016931-02S2

PS 2122 CTR1, BUT LESS LIKELY DMT1, PLAYS A CRITICAL ROLE IN MANGANESE (MN)-INDUCED COPPER (CU) ACCUMULATION IN THE BLOOD-CSF BARRIER.

G. Zheng and W. Zheng. *School of Health Sciences, Purdue University, West Lafayette, IN.*

The blood-cerebrospinal fluid barrier (BCB) in the choroid plexus plays a key role in maintaining Cu homeostasis in the brain. Previous studies from this lab show that Mn exposure alters cerebral Cu homeostasis, which may underlie the development of neurodegenerative manganism. Cu transporter-1 (Ctr1) and divalent metal transporter-1 (DMT1) are known to transport metals; however, their roles in Cu transport in BCB under Mn exposure have not been defined. This study investigated the role of Ctr1 and DMT1 in Cu uptake in the choroidal Z310 cells. Mn exposure (100 μM) for 24 hr increased the 64Cu uptake by the cells, and up-regulated the protein levels of both Ctr1 and DMT1. Ctr1 knockdown using Ctr1 siRNA before Mn exposure diminished the Mn-induced increase of 64Cu uptake. Unexpectedly, however, knocking down DMT1 by DMT1 siRNA, followed by Mn exposure, resulted in an increase of the cellular [64Cu] uptake. To distinguish the exact role of these two transporters, we established Z310 cell-based tetracycline (Tet)-inducible Ctr1 or DMT1 expression cell lines that carry Tet repressor (TetR). When Tet is added to the culture medium and binds to the TetR, the transcription of Ctr1 or DMT1 gene proceeds, leading to the gene overexpression. The Ctr1- or DMT1- inducible cells were treated with 100 μM Mn for 24 hr and then incubated with Tet for another 6 hr, followed by 64Cu uptake assay. Results showed that Tet-induced expression of Ctr1 further increased 64Cu uptake in Ctr1-inducible cells; over-expression of DMT1, however, did not increased 64Cu uptake in DMT1-inducible cells. Taken together, these data indicate that Mn exposure results in an increased cellular Cu uptake and increased protein expression of both Ctr1 and DMT1 in choroidal Z310 cells. It is Mn-induced upregulation of Ctr1, but less likely DMT1, that prompts Cu uptake by the choroidal epithelial cells. Unlike the role of DMT1 in Mn-Fe interaction, Ctr1 appears to play a critical role in response to Mn exposure and to regulate cellular Cu uptake. (Supported by NIH/NIEHS RO1-ES008146)

PS 2123 EXAMINING A POTENTIAL GENE-ENVIRONMENT INTERACTION BETWEEN THE PD-ASSOCIATED GENE *PARKIN* AND MANGANESE IN A *C. ELEGANS* MODEL.

S. Chakraborty[1] and M. Aschner[2, 3]. [1]*Neuroscience Graduate Program, Vanderbilt University, Nashville, TN,* [2]*Pediatrics, Vanderbilt University, Nashville, TN and* [3]*Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN.*

Manganese (Mn) is a trace element that serves as a vital micronutrient in human diet. However, overexposure from the environment can lead to a toxic condition known as Manganism. This disorder shares similar neuropathology with Parkinson's disease (PD), implicating dopaminergic (DAergic) cell loss as the primary target. Both manganism and PD lead to neurological and motor deficits, along with increased mitochondrial dysfunction. The mechanisms behind these disorders are still not clearly understood. Of recent interest are methods to elucidate a potential gene-environment interaction, using *C. elegans* as a genetic model that contains the necessary DAergic machinery. Many genes associated with PD have been identified in the literature, including the *parkin/PARK2* (*pdr-1* in worms) gene that encodes for an E3-ubiquitin ligase protein that is also involved in inducing mitophagy. It has been previously shown that Mn toxicity exhibits specificity to DAergic cells, and that parkin confers protection to these cells from Mn exposure *in vitro*. We, therefore, hypothesized that a loss-of-function mutation in *parkin* increases the vulnerability of *C. elegans* to Mn toxicity. Synchronous L1 worms from wildtype N2 and *pdr-1* mutant strains were acutely treated with a range of Mn doses (0-200 mM $MnCl_2$) for 30 minutes, followed by lethality scoring of approximately 200 worms 24 hours post-treatment. Using transgenic worms with a *pdr-1* deletion, we show that a loss in *parkin* increases sensitivity to Mn toxicity (LD_{50}=33.64 mM $MnCl_2$) compared to wild-type N2 worms (LD_{50}=59.27 mM $MnCl_2$), as seen with a leftward shift in the dose-response curve. This data indicate that parkin is protective against Mn toxicity *in vivo*, with loss of parkin potentially producing increased oxidative stress from impaired mitophagy.

PS 2124 HSPS ARE IMPORTANT MEDIATORS OF MN-INDUCED TOXICITY IN *C. ELEGANS.*

D. Avila and M. Aschner. *Pediatrics/Toxicology, Vanderbilt University, Nashville, TN.*

Heat shock proteins (HSPs) are essential for survival under conditions of acute stress. Particularly, HSPs are protective in Alzheimer's and Huntingtons' disease. Furthermore, it has been hypothesized that these chaperones act in association with DJ-1 and PINK-1, translocating these proteins to the mitochondria in order to exert their antioxidant response. Mutations in the genes that codify DJ-1 and PINK-1 are associated with early-onset Parkinson's disease, a neurodegenerative disease that impairs the motor system because of degeneration of dopamine (DA)-producing neurons. Similarly, manganese (Mn) poisoning also causes degeneration of DAergic neurons by causing mitochondrial dysfunction, oxidative stress and motor defects. We have previously observed that DJ-1 and PINK -1 are highly expressed after Mn exposure in Caenorhabiditis elegans, probably in an attempt to protect the cells against the damaging mitochondrial effects of this metal. Considering the putative importance of HSP to DJ-1 and PINK-1 function, we hypothesized that loss-of-function of hsp would impair their translocation to mitochondria, increasing Mn toxicity in C. elegans. N2 wild type worms and hsp knockout worms (15 strains) were maintained as standardized conditions. Synchronous L1 worms were

exposed for 30 min to MnCl2 (0-200mM). Proteins for protein oxidation analysis and RNA for RT-PCR were obtained immediately after exposure. In parallel experiments, survival was scored after 24h exposure. We observed that 4 of the hsp mutant strains tested were more sensitive to Mn when compared to N2 (hsp-1 (tm2318), hsp-3 (ok1083), chn-1 (by155), hsf-1 (sy441)). Furthermore, we noted that increased protein oxidation was associated with increased sensitivity to Mn. Using RT-PCR, we observed that the expression of pink-1 and dj-1 are altered by Mn and that these alterations are distinct in the mutants in comparison to wild type worms. Our results indicate that loss-of- function of hsp increases Mn toxicity and that DJ-1 and PINK-1 likely play an important role in protecting against this toxicity by mediating levels of HSPs. Supported by R01 ES105663.

PS 2125 THE EFFECTS OF MANGANESE ON MITOCHONDRIAL ACONITASE AND CYTOCHROME C OXIDASE IN THE GILL OF THE BIVALVE *CRASSOSTREA VIRGINICA.*

K. Huang, A. Hutchinson, H. Huang, Z. Nicholas, R. Desroches, E. J. Catapane and M. A. Carroll. *Biology, Medgar Evers College, Brooklyn, NY.*

Manganese (Mn) is an essential trace metal but excessive exposure can lead to Mn toxicity. In humans Mn is neurotoxic and can lead to Manganism, a Parkinsons-like disorder. It is hypothesized that Mn toxicity causes mitochondrial dysfunction. Mitochondrial dysfunction is recognized as a factor in aging and many neurodegenerative diseases including Alzheimer's and Parkinson's disease. Being a redox-active element, excess Mn can increase mitochondrial oxidative stress and impair activities involved in energy homeostasis. Previously we showed Mn accumulates in gill and other tissues of the bivalve, *Crassostrea virginica* and Mn treatments selectively disrupt the dopaminergic system controlling lateral cilia in gill. We also showed it impairs gill mitochondrial respiration. In this study we examined effects of Mn treatments on two mitochondrial enzymes: aconitase, a Kreb Cycle enzyme and cytochrome c oxidas, complex IV of the respiratory chain. Gill mitochondria were prepared from adult *C. virginica* and aliquots of mitochondrial suspension were exposed to various concentration of Mn. Mitochondrial aconitase and cytochrome c oxidase activities were determined spectrophotometrically. Mn (up to 50 Mn) was found to inhibit aconitase, but not cytochrome c oxidase activity. The results corroborate our previous findings that Mn disrupts mitochondrial function in oyster gill and further demonstrates a mechanism by which Mn can disrupt energy homeostasis by impairing aconitase, an oxidative stress-sensitive enzyme of the Kreb Cycle. This work was supported by 2R25GM06003 of the Bridge Program of NIGMS, 0516041071 of NYSDOE and 0622197 of the DUE Program of NSF.

PS 2126 MN^{2+} INHIBITION OF OXIDATIVE PHOSPHORYLATION IN LIVER, BRAIN, AND HEART MITOCHONDRIA.

T. E. Gunter[1], B. Gerstner[1], C. Gavin[2] and K. Gunter[1]. [1]*Biochemistry and Biophysics, University of Rochester School of Medicine, Rochester, NY and* [2]*Pfizer, Groton, CT.*

Manganese (Mn) induces neurotoxicity primarily within the basal ganglia and is known to decrease ATP production via inhibition of oxidative phosphorylation (ox phos). We hypothesize that Mn inhibition of ox phos represents an initial insult to the target tissue, resulting in functional damage that ultimately produces the signs and symptoms of Mn toxicity. We have used State 3 (rapid) oxidation rate assays using an oxygen electrode to explore sites of Mn inhibition of ox phos in isolated liver, brain, and heart mitochondria. This approach is appropriate for the energetically active target tissue of the globus pallidus and striatum and allows us to identify the Mn inhibited steps which actually affect the ATP production rate. It also reveals important tissue differences in patterns of Mn inhibition. The results show that Mn^{2+} inhibits Ca^{2+} activation of αketoglutarate dehydrogenase, but this inhibition does not decrease the rate of ox phos. They also verify that Mn^{2+} inhibits ox phos with different patterns in liver, brain, and heart mitochondria. In liver and heart mitochondria the primary site of inhibition is the F_1F_0 ATP synthase, whereas in brain mitochondria Mn^{2+} inhibition affects two independent loci. Mn^{2+} inhibits ATP production fueled by either glutamate plus malate or succinate two to three times more strongly in brain mitochondria than in liver or heart mitochondria. When succinate is the substrate, the primary site of Mn^{2+} inhibition in brain mitochondria is either fumarase or complex II; when glutamate plus malate are the substrates, the primary site of Mn^{2+} inhibition is likely either the aspartate/glutamate exchanger or aspartate transaminase. This work was supported by NIH ES10041 and by Dept. of Defense (MHRP) W81XWH-05-1-0239.

PS 2127 MECHANISM OF ENHANCED HEME OXYGENASE-1 (HO-1) ACTIVITY AND THE ROLE OF HO-1 IN CONTROLLING INFLAMMATORY CYTOKINE OUTPUT IN LPS-STIMULATED MICROGLIA.

C. A. Dodd, I. I. Georgieva and N. M. Filipov. *Physiology and Pharmacology, University of Georgia, Athens, GA.*

Accumulating evidence suggests that overexposure to manganese (Mn) increases output of glial-derived inflammatory products which indirectly contribute to the neurotoxic effects of this essential metal. Specifically, in microglia, Mn induces release of reactive oxygen species (ROS), namely H_2O_2 and potentiates lipopolysaccharide- (LPS) induced release of inflammatory cytokines (TNF-α, IL-6, and IL-1β) and nitric oxide. In addition, we previously observed that the Mn-potentiated increase of cytokine output in microglia is accompanied by markedly increased expression of heme oxygenase-1 (HO-1). As the enzyme responsible for heme catabolism, the expression of HO-1 is typically upregulated in response to oxidative stress. However, the mechanism of Mn-potentiated HO-1 and its role in the inflammatory response has yet to be defined. The present study examined both the longevity of Mn-enhanced cytokine output (TNF-α and IL-6) and the role of Mn-potentiated HO-1 expression along with possible mechanisms of HO-1 induction. After 24 h, Mn (100 μM) potentiated TNF-α and IL-6 at both protein and mRNA level. Upon further investigation, pharmacological inhibition of HO-1 activity decreased Mn+LPS-induced cytokine output and Mn-induced H_2O_2 output unveiling both an anti-inflammatory and antioxidant role for the increase of HO-1 caused by Mn+LPS. At the molecular level, the mechanism of HO-1 potentiation by Mn appears to be related to an increased nuclear presence of small Maf proteins, which can increase HO-1 transcription by forming heterodimers with nuclear factor E2-related factor-2 (Nrf-2). Future studies, using transcription inhibitor actinomycin D, will examine Mn effects on cytokine mRNA stability and the role of HO-1 in the process. This would determine whether potentiating effect of Mn on microglial inflammatory cytokine output is due to enhanced mRNA stability, increased mRNA production, or both. Supported by: R01ES016965 (NIEHS).

PS 2128 OXIDATIVE INJURY AND EP2 SIGNALING IN MANGANESE NEUROTOXICITY.

D. Milatovic[1], Y. Yu[1], S. Zaja-Milatovic[2] and M. Aschner[1]. [1]*Pediatrics, Vanderbilt University, Nashville, TN and* [2]*Medicine, Vanderbilt University, Nashville, TN.*

Manganism is caused by neuronal injury in both cortical and subcortical regions, particularly the basal ganglia. The basis for the selective neurotoxicity of Mn remains incompletely understood, yet several studies suggest that inflammatory processes and oxidative damage play prominent roles in the degeneration of dopamine-containing neurons. We and others have associated increase in proinflamatory prostaglandins with neuronal oxidative damage in different models of neurodegeneration. Notably, we have identified that prostaglandins E2 (PGE2) signaling through EP2 receptor promotes inflammatory oxidative stress in the lipopolysaccharide (LPS) model of inflammation. Since oxidative stress and neuronal injury are shared by varied neurodegenerative disease, we investigated if PGE2 and its EP receptor subtype are also critical regulators of cerebral oxidative damage and neurodegeneration in Mn neurotoxicity. Results from our study showed significant ($p<0.05$) increase in cerebral biomarkers of oxidative damage, F2-isoprostanes (F2-IsoPs) and pro-inflammatory PGE2 (145% and 127%, respectively) along with progressive spine degeneration and dendritic damage of striatal medium spiny neurons (MSNs) of mice exposed to Mn (100 mg/kg, sc). Importantly, our in vivo experiments with the transgenic mice showed that lack of EP2 completely blocked Mn-induced cerebral F2-IsoP formation and mediated, at leat in part Mn-induced synaptodendritic damage of MSNs. Elucidation of molecular mechanisms underlying onset and progression of Mn-induced neuronal damage will be instrumental in the development of disease modifying therapeutic strategies (Supported by DoD W81XWH-05-0239 and NIH ES R01 10563).

PS 2129 GLIAL INTERACTIONS AND NEUROINFLAMMATION IN MANGANESE NEUROTOXICITY.

K. Sullivan[1] and R. Tjalkens[1, 2]. [1]*Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO and* [2]*Molecular and Cellular Biology, Colorado State University, Fort Collins, CO.*

Microglia are the resident immune cells of the brain; however, chronic activation of microglia can lead to inflammatory insults due to the production of proinflammatory mediators. Previous studies have implicated activated microglia and astrocytes in neurodegenerative disorders of the basal ganglia such as Parkinson's disease and

manganism. Recent work from our laboratory identified activated microglia expressing inducible nitric oxide (iNOS) in the basal ganglia of mice exposed to manganese (Mn) before the appearance of activated astrocytes; however, the role of activated microglia in manganism is poorly understood. In this study we postulated that Mn directly activates microglia, resulting in increased expression of tumor necrosis factor α (TNF- α), interleukin 1-beta (IL-1β) and iNOS that ultimately lead to increased astrocyte activation. Primary microglia and astrocytes were isolated from C57Bl/6 mice in mixed cultures then purified for experiments. Immunofluorescence staining for microglia and astrocytes was performed using ionized calcium binding adaptor protein-1 (IBA-1) and glial fibrillary acidic protein (GFAP), respectively. This method yielded culture purities of 97% for microglia and 99% for astrocytes. Treatment of microglia with Mn induced a dose-dependent expression of TNFα, iNOS mRNA and protein. Furthermore, a quantitative PCR array revealed increased expression of proinflammatory mediators in microglia when treated with Mn. Treatment of astrocytes with conditioned media from Mn-treated microglia or via co-culture with microglia caused an activated phenotype characterized by increased iNOS, TNFα, and IL-1β expression. Collectively, these data indicate that Mn activates microglia in a dose-dependent manner resulting in increased production of proinflammatory mediators that enhance activation of astrocytes, suggesting a complex pattern of glial-glial interactions underlying a neuroinflammatory phenotype in this model.

PS 2130 NOVEL NADPH OXIDASE MODULATOR PROTECTS AGAINST NEUROINFLAMMATION ASSOCIATED WITH MANGANESE NEUROTOXICITY.

C. Hogan[1], R. Gordon[1], A. Kanthasamy[1], V. Anantharam[1], B. Kalyanaraman[2] and A. G. Kanthasamy[1]. *[1]Biomedical Sciences, Iowa Center for Advanced Neurotoxicology, Iowa State University, Ames, IA and [2]Biophys, Medical College of Wisconsin, Milwaukee, WI.*

Sustained neuroinflammation has become increasingly evident as a causative factor in the progression of many neurodegenerative diseases including Parkinson's disease (PD). Exposure to environmental neurotoxicants such as metals and pesticides are considered as potential risk factors in the pathogenesis of PD. Microglia are a large part of the innate immune system of the CNS and are capable of producing rapid neuroinflammatory responses when confronted with pathogens or neurotoxic insults. Metal exposure has been shown to trigger activation of both microglia and astroglia in order to counter neurotoxic insults. Recently, studies have shown that manganese (Mn) exposure by itself or in combination with other inflammatory stimuli can produce neuroinflammatory responses in the nigrostriatal dopaminergic system. We and others have shown that Mn can augment lipopolysaccharide (LPS)-induced cytokine production, iNOS activation, and ROS generation in microglial cells. In the present study, we sought to investigate the anti-inflammatory potential of a novel NADPH oxidase inhibitor MIA-4025 against microglial activation in response to Mn/LPS-induced inflammatory events. MIA-4025 treatment significantly blocked Mn/LPS-induced iNOS activation and ROS generation in both primary microglia and BV2 microglia cell models. Measurement of cytokine release in the supernatant of MIA-4025 treated BV2 cell lines and primary microglia cells revealed that the compound effectively suppressed the release of TNFα, IL-1β, IL-6, IL-10 and IL-12. Expression of phox proteins, components of the NADPH oxidase complex and a main contributor to ROS production, was also decreased with MIA-4025 treatment. Collectively, these results suggest that our translational approach with MIA-4025 can suppress neuroinflammatory events associated with neurotoxic metal exposure. (Supported by NIH grants ES10586, NS065167 & NS039958.)

PS 2131 PROTEIN KINASE C IS INVOLVED IN MANGANESE-MEDIATED DISRUPTION OF GLUTAMINE TURNOVER IN ASTROCYTES.

M. Sidoryk-Wegrzynowicz[1], E. Lee[2] and M. Aschner[1]. *[1]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN and [2]Department of Physiology, Meharry Medical College, Nashville, TN.*

Manganese (Mn) is an essential trace element for normal human development and is required for proper functioning of a variety of physiological processes. Chronic exposure to Mn can cause manganism, a neurodegenerative disorder similar to Parkinson's disease. Mn neurotoxicity is characterized by impairment both in the expression and activity of glutamine (Gln) transporters in astrocytes. Since protein kinase C (PKC) activation leads to downregulation in a number of neurotransmitter transporters and Mn increases PKC activity, we hypothesized that the PKC signaling pathway may contribute in Mn-mediated disruption of glutamine turnover. We have found that Mn exposure elevates phosphorylation of PKCα and PKCδ isoforms. PKCs activity (measured by phosphorylation of substrate peptide using radiolabeled ATP) was found to be increased after Mn treatment. As was previously observed, Mn caused a decrease in Gln uptake. This effect was blocked by PKCs inhibitor. Notably, PKC activation by phorbol 12-myristate 13-acetate (PMA) caused a decrease in Gln uptake mediated by systems ASC and N, and had no effect on systems A and L. Treatment with PMA significantly decreased SNAT3 (system N) and ASCT2 (system ASC) protein levels. Additionally, a co-immunoprecipitation study showed association of SNAT3 and ASCT2 with the PKCδ isoform. Combined, these findings demonstrate that Mn-induced dysregulation of Gln homeostasis in primary astrocytes involves PKC signaling.
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PS 2132 EXPRESSION OF MUTANT HUNTINGTIN INCREASES ARGINASE ACTIVITY IN MOUSE STRIATUM, BUT DECREASES ITS SUSCEPTIBILITY TO MANGANESE EXPOSURE.

M. Wegrzynowicz[1, 2], H. K. Holt[1, 2] and A. B. Bowman[1, 2, 3]. *[1]Department of Neurology, Vanderbilt University Medical Center, Nashville, TN, [2]Vanderbilt Kennedy Center, Vanderbilt University Medical Center, Nashville, TN and [3]Center for Molecular Toxicology, Vanderbilt University Medical Center, Nashville, TN.*

Huntington disease (HD), a neurodegenerative disorder principally affecting the striatum, is caused by mutation in the huntingtin (Htt) gene, but severity of the disease may be strongly influenced by unidentified environmental factors. We have reported a gene-environment interaction wherein expression of mutant (mt) HTT impairs striatal manganese (Mn) accumulation. We hypothesize that mtHTT expression may alter cellular markers of Mn toxicity.
When testing potential markers we found that basal activity of the Mn-dependent enzyme, arginase, is elevated in the homogenates of striata from mtHTT-expressing mice (YAC128Q) comparing to wild-type (wt) littermates. Additionally, we observed that *in vivo* exposure to Mn (three 50 mg/kg $MnCl_2$ subcutaneous injections over one week) strongly elevates enzyme activity in striatal extracts, however this effect is less pronounced in mutant mice. Cortical activity was lower than striatal, and no effect of genotype was found. *In vitro* supplementation of vehicle-exposed mice striatum homogenates with 2 μM Mn (a concentration exceeding the values measured in the homogenates from animals exposed to Mn *in vivo*) was much less effective in arginase activation than subcutaneous exposure *in vivo*. We conclude that increased activity of arginase, an enzyme known to be neuroprotective, may be a compensatory response to mtHTT cytotoxicity in the striatum. Impaired activation of striatal arginase by Mn administered *in vivo* in YAC128Q mice is probably due to a mtHTT-induced deficit of Mn accumulation. Interestingly, the effect of Mn is most likely only partially mediated by increased availability of Mn, a cofactor of arginase. Inability of *in vitro* added Mn to replicate the effects of *in vivo* exposure suggests that Mn may induce some cellular processes contributing in the activation of arginase.
Support: NIH ES016931

PS 2133 NEUROTOXIC EFFECTS OF INTRANASAL MANGANESE EXPOSURE AND INTERACTION WITH CADMIUM EXPOSURE IN A MOUSE MODEL.

A. H. Moberly, J. Pottackal, L. Czarnecki, T. Rubinstein, D. J. Turkel and J. P. McGann. *Psychology Department, Rutgers University, Piscataway, NJ.*

Exposure to aerosolized manganese occurs in a variety of industrial settings, including mining and welding, but its effects on neurons directly exposed to manganese has not been explored in vivo. Transgenic mice expressing the fluorescent exocytosis indicator synaptopHluorin (spH) in olfactory receptor neuron terminals were used to visualize odorant-evoked neurotransmitter release from the olfactory nerve following acute toxicant exposure. Mice received a unilateral intranasal instillation of 2, 20, or 200 μg manganese chloride and a vehicle control on the contralateral side. Two days later they were anesthetized, implanted with a cranial window overlying the olfactory bulbs, and odorant-evoked neurotransmitter release was quantified bilaterally via optical imaging of spH signals. At the highest dose, odorant-evoked sensory input to the olfactory bulb on the Mn-exposed side was reduced to 14% of control and while epithelial projections to the olfactory bulb were slightly reduced. The 20 μg dose reduced responses to 58% of control, while the lowest dose had no significant effect. Because we have previously shown similar effects using intranasal cadmium exposure, we further hypothesized that mixtures of the two heavy metals might demonstrate an interaction. To test this, the above experiment was repeated using instillations of a combination of 20 μg Mn and 2 μg Cd, doses found to produce intermediate reductions in odorant-evoked responding when presented individually. Mice exposed to this mixture exhibited odorant-evoked transmitter release from the olfactory nerve equal to only 11% of control. This decrease suggests that peripheral exposure to cadmium and manganese can cause pathopysiology comparable to the sum of their effects individually. We conclude that intranasal Mn exposure can significantly disrupt olfactory function. Moreover, its neurotoxic effects can summate with those of Cd, suggesting that total exposure to both should be considered in assessing risk levels.

PS 2134 MANGANESE AND VANADIUM EXPOSURE INDUCES SEVERE NEUROTOXIC RESPONSE IN OLFACTORY SYSTEM OF AN ANIMAL MODEL: POTENTIAL RELEVANCE TO OLFACTORY DYSFUNCTION AND PARKINSON'S DISEASE (PD).

H. Afeseh Ngwa, A. Kanthasamy, V. Anantharam and A. G. Kanthasamy. *Iowa State University, Ames, IA.*

Chronic environmental exposure to heavy metals through occupational and industrial sources has been suggested in the etiopathogenesis of neurodegenerative conditions like PD. Metal exposure often occurs with multiple rather than individual components, warranting an understanding of the neurotoxic effects of metal mixtures. Vanadium pentoxide (V2O5) typically presents along with manganese (Mn) especially in welding rods. Mechanisms of V2O5/Mn interaction and resulting consequences of neurotoxicity are unknown. Using cell culture models, we have recently shown that the V2O5/Mn metal mixture is a more potent neurotoxicant than V2O5 or Mn alone. In this study, we examined the neurotoxic effects of Mn, V2O5 and Mn - V2O5 co-exposure in an animal model. C57 black mice were intranasally exposed to 252μg MnCl2 alone, 182μg V2O5 alone, or MnCl2-V2O5 in combination three times a week for up to one month. The delivered metal levels are relevant to the exposure limits found in many workplace settings. Following exposure, behavioral, neurochemical, and histological studies were performed. Our results revealed dramatic decreases in olfactory bulb weights and levels of tyrosine hydroxylase, dopamine and DOPAC in the treatment groups as compared to control. Interestingly, increased levels α-synuclein in the substantia nigra of treated animals were observed. We also observed accompanying locomotor deficits and olfactory dysfunction with the co-treatment group producing the most severe deficits. Taken together with recent observations of anosmia in PD patients, our results suggest that exposure to Mn and V2O5 can adversely affect the olfactory and nigral systems, with accompanying behavioral deficits. These results also indicate that mixtures of Mn and vanadium might have more serious neurotoxic implications in environmentally linked PD like conditions than that of individual metals (NIH grant ES10586).

PS 2135 CHRONIC MANGANESE ALTERS THYROID AND ESTROGEN LEVELS IN RATS BLOOD.

O. Soldin[1] and M. Aschner[2]. [1]*Departments of Oncology, Medicine, Physiology and Biophysics, Obstetrics and Gynegology, Georgetown University, Washington, DC and* [2]*Departments of Pediatrics and Pharmacology, Vanderbilt University, Nashville, TN.*

Manganese (Mn) is an essential trace nutrient that is potentially toxic at high levels of exposure. As a constituent of numerous enzymes and a cofactor, manganese plays an important role in a number of physiologic processes in mammals. Exposure to high Mn levels can result in manganism characterized by motor deficits and damage to nuclei of the basal ganglia and dopaminergic deficits. Dopamine is a known inhibitory modulator of thyroid stimulating hormone (TSH) secretion. The involvement of dopamine and dopaminergic receptors in neurodevelopment, as well as TSH modulation, led us to hypothesize that excessive manganese exposure may lead to the disruption of normal thyroidal processes and thyroid hormone synthesis. Four groups of rats were treated for 14 weeks. The control group received normal dietary levels of Mn. A second group received a 10-fold excess of Mn daily. In addition two other groups received an iron (Fe) deficient diet (6 mg/kg/day) in the presence of normal or excess of Mn. Serum thyroxine (T4), triiodothyronine (T3) and a panel of 14 steroids were measured using liquid chromatography tandem mass spectrometry (LC/MS/MS) at the end of exposures. Mn exposure resulted in a decrease in T4 and T3 when rats were fed iron deficient diets. Similar results were obtained for Cortisol, DHEAS and progesterone. These results suggest that chronic Mn exposure may alter thyroid and steroid hormone levels, which may have critical implications for normal growth and differentiation and fetal neurodevelopment during pregnancy. Supported by DoD W81XWH-05-0239, NIEHS 10563, the Flight Attendants Medical Research Institute and the Georgetown Bioanalytical Core Laboratory.

PS 2136 PEPTIDOME AND PROTEOME IN MURINE CHOROID PLEXUS-CEREBROSPINAL FLUID SYSTEM: EFFECT OF CHRONIC MANGANESE (MN) EXPOSURE.

G. J. Li[1], K. H. Wei[2], H. M. Jing[1], F. Yang[2], C. Y. Zhao[1], L. Ma[1], T. Zhang[2], X. Y. Zhang[2], J. Z. Liu[1], L. P. Liu[1] and W. Zheng[3]. [1]*Beijing Center for Preventive Medicine, Beijing, China,* [2]*Beijing Proteome Research Center, Beijing, China and* [3]*Purdue University, West Lafayette, IN.*

Occupational Mn exposure is known to cause Parkinsonism; yet the mechanism is unclear. The choroids plexus (CP)-cerebrospinal fluid (CSF) system maintains the homeostasis of brain's milieu including Mn. This study was designed to explore the normal peptidome and proteome in the CP, and the change of proteome in the CP-CSF system after Mn exposure. Rats (1.5 mn) were divided into 3 groups; each received daily ip injections of either MnCl2 (6 mg Mn/kg) or saline (as controls) for 30, 90 days, or 90 days followed by additional 30-day convalescence. The CP and CSF were collected and analyzed. The results showed: (1) 163 peptides in the normal CP were identified by using matrix-assisted laser desorption/ionization TOF/TOF mass spectrometry and nano-LC-MS/MS. (2) A total of 231 non-redundant proteins were identified in the CP by two ways of 1-DE combining with LC-Q-TOF-MS and 1-DE combining with LC-IT-MS. Further analysis by Gene Ontology (GO) annotation revealed that the proteins responsible for binding, transport and structural integrity accounted for 67% of all the identified proteins in the CP. (3) In the CP of Mn-treated rats, a total of 448 protein spots were isolated by 2D-PAGE, and 69 protein spots were significantly different from the controls by using PDQuest 8.0.1 software for quantitative analyses (changes more than 10 folds). (4) Moreover, a group of 32 differentially expressed proteins in the CP of Mn-exposed rats were identified by Nano-LC-MS/MS, of which 27 were up-regulated (e.g. mitochondrial aconitase, nucleobindin-1 precursor), and five were down-regulated (e.g. transthyretin). Based on the GO, these proteins involve largely in the functions of binding, catalysis and transportation. An in-depth study of Mn-related peptidome and proteome in the CSF is currently in progress. (PRC Beijing City Personnel Grant 2007-62; US NIH/NIEHS ES017055)

PS 2137 REDUCED COPPER CLEARANCE BY THE BLOOD-CSF BARRIER FOLLOWING *IN VIVO* MANGANESE AND THE ROLE OF CU TRANSPORTER ATP7A.

X. Fu, Y. Zhang, W. Jiang, A. D. Monnot and W. Zheng. *School of Health Sciences, Purdue University, West Lafayette, IN.*

Previous animal and human studies by this lab showed a significant increase of Cu in blood, saliva and brain following Mn exposure. However, the mechanism is unknown. ATP7A functions to translocate Cu from the cytosol to cellular membrane. This study was designed to test the hypothesis that Mn exposure interfered the intracellular trafficking of ATP7A in the choroid plexus (CP) where the blood-CSF barrier (BCB) resides. Rats received i.p. injection of 6 mg Mn/kg as MnCl2 or saline, 5 d/week for 4 weeks. The ICP-MS analyses revealed a significant increase of Mn and Cu levels in serum, cerebrospinal fluid (CSF), CP, hippocampus, and frontal cortex, when compared with controls. An in situ ventriculo-cisternal perfusion study by infusing the mixture of ^{64}Cu and a space marker ^{14}C-sucrose showed that Mn exposure did not affect the CSF clearance of ^{14}C-sucrose, but it significantly increased, about 2.6 folds, ^{64}Cu radioactivity in the CSF as compared to controls, suggesting a reduced Cu clearance by the BCB. The mRNA expression of ATP7A in choroidal epithelial Z310 cells was significantly higher in Mn-treated (100 μM for 48 hr) groups than in controls. Confocal imaging studies also exhibited an increased fluorescent signal in choroidal epithelial Z310 cells after Mn treatment. Additionally, the confocal technique was used to study the intracellular trafficking of ATP7A in freshly isolated CP tissues from in vivo Mn exposed rats. An evident intracellular migration of ATP7A from the sub-nucleus region to the apical membrane in the choroidal epithelia, which are in the direct contact with the CSF, was observed. Collectively, these data suggest that Mn exposure results in the overload of intracellular Cu with an ensuing increase of ATP7A level in the BCB. A subsequent translocation of ATP7A towards the apical microvilli facing the CSF may expel Cu into the CSF and reduce Cu clearance from this central milieu. Consequently, an increased Cu in the CNS may account for Mn-induced neurotoxicity. (Supported by NIH/RO1-ES008146)

PS 2138 INCREASED BETA-AMYLOID DEPOSITION IN TRANSGENIC APP MICE FOLLOWING CHRONIC LEAD (PB) EXPOSURE.

W. Zheng[1], H. Gu[2], X. Wei[2], A. D. Monnot[1] and Y. Du[1, 2]. [1]*School of Health Sciences, Purdue University West Lafayette, IN and* [2]*Neurology, Indiana University School of Medicine, Indianapolis, IN.*

Pb is an environmental toxicant that may contribute to the etiology of Alzheimer's disease. Our early studies suggest that Pb exposure reduces the clearance of beta-amyloid (Aβ) from the cerebrospinal fluid (CSF) by acting on Aβ removal system in the choroid plexus (CP). This study tested the hypothesis that a reduced Aβ clearance following Pb exposure increased Aβ levels in the CSF, leading to amyloid plaque formation in brain tissues. APP transgenic mice were used for their ability to overexpress human amyloid precursor protein (APP) with an age-related deposition of neuritic plaques beginning at 3 mn of age. Following acute Pb exposure (27 mg Pb/kg, ip, 24 h) in 2-mn-old mice, ELISA revealed a significantly higher level of

Aβ1-40 in CSF and hippocampus in Pb-treated mice than in controls (p<0.05); the level of Aβ1-42 was also higher in hippocampus. Chronic Pb exposure (27 mg Pb/kg, oral gavage, 2 mn) in 2-mn-old mice showed a significant increase of Aβ1-40 in the CSF as compared to controls. Histopathological examination of brain sections further revealed that Pb treatment increased the amount of the condensed amyloid plague in hippocampus region; however, the onset of the plague formation was not evidently shortened in these mice. When the primary mixed culture of cortical and hippocampal neurons derived from APP mice was exposed to Pb (1-10 uM, 24 h), the level of total Aβ was not changed. Interestingly, the levels of low-density lipoprotein receptor-related protein-1 (LRP1), an Aβ degradation enzyme, in the CP of acute Pb-exposed mice were significantly reduced. These data suggest that both acute and chronic Pb exposures in APP mice increase the levels of Aβ in the CSF and brain tissue, which leads to the formation of amyloid plagues in brain tissues. The increased level of Aβ is not the result of overproduction of Aβ in the neuron, but appears to be mediated, at least in part, by Pb-induced inhibition of LRP1 expression in the choroid plexus. (Supported by NIH/NIEHS ES017055).

PS 2139 NEUROIMAGING OF MANGANESE TOXICITY: EFFECT OF EXPOSURE TIME ON ACCUMULATION IN HUMAN BRAIN.

J. Xu[1, 2], X. Li[3], S. Streitmatter[1], L. Long[3], W. Zheng[1], Y. Jiang[3] and U. Dydak[1, 2]. *[1]School of Health Sciences, Purdue University, West Lafayette, IN, [2]Indiana Univeristy School of Medicine, Indianapolis, IN and [3]Guangxi Medical University, Nanning, China.*

Manganese (Mn) accumulation in the brain can be visualized by signal hyperintensities in T1-weighted MRI. We used 1mm isotropic 3D T1-weighted Magnetic Resonance Imaging (MRI) at 3.0T and inversion recovery (IR) MRI to a) analyze the human brain regions showing Mn accumulation and b) to study the dependence of the T1 relaxation time in the globus pallidus (GP) on exposure duration. Nine smelters, eight welders and ten controls were recruited from two Mn-Fe alloy manufactures in China. Mn deposition in the GP was quantified using three approaches: The T1 intensity ratio between GP and frontal white matter (PIwm), the T1 intensity ratio between GP and neck muscle (PImu) and T1, calculated from the IR images. Our results show that (1) T1 hyperintensities typical of Mn accumulation were most prevalent in the GP, followed by depositions in substantia nigra pars recticularis, the tegmentum of the midbrain, subthalamic nucleus, pineal stalk and optic chiasm. In cases of high Mn accumulation additional signal changes in substantia nigra pars compacta, pituitary gland, olfactory bulb and red nucleus were found. (2) Both smelters and welders had significantly reduced T1 values in the GP compared to controls (p = 0.007 and p < 0.0001, respectively), and a significant difference was also found between welders and smelters (p=0.0006). (3) More interestingly, the T1 values were reduced as a function of years of exposure in the welders (R=0.935, p<0.01). (4) The fact that only the PImu (p=0.03) but not the PIwm (p=0.3) could distinguish the two exposed groups appeared to be a further indication that Mn accumulates throughout the brain, including in frontal white matter. Overall our study shows that a wide range of brain areas are affected by Mn accumulation and suggests that the T1 relaxation time is a more sensitive measure of brain Mn accumulation than the commonly used pallidal index. (Supported by NIH/NIEHS ES017498).

PS 2140 NEUROIMAGING OF MANGANESE TOXICITY: GABA AND METABOLIC CHANGES IN THE HUMAN BRAIN.

Z. Long[1, 2], Y. Jiang[3], X. Li[3], J. Xu[1, 2], L. Long[3], W. Zheng[1] and U. Dydak[1, 2]. *[1]School of Health Sciences, Purdue University, West Lafayette, IN, [2]Indiana University School of Medicine, Indianapolis, IN and [3]Guangxi Medical University, Nanning, China.*

Manganese (Mn) overexposure is known to cause neurological, psychological and motor abnormalities. It is suggested that Mn may perturb neurotransmitter homeostasis and cause oxidative stress. In this study we used Magnetic Resonance Spectroscopy (MRS) to determine *in vivo* concentrations of γ-aminobutyric acid (GABA), glutathione (GSH), taurine (Tau), myo-inositol (mI), and glycerylphosphorylcholine (GPC) in three volumes of interest: (a) centered on the thalamus, but also containing adjacent basal ganglia tissue, (b) in posterior cingulated cortex (PCC) and (c) in hippocampus. Nine smelters, eight welders and ten controls from two Mn-Fe alloy manufactures in China were scanned using a 3.0T scanner. A GABA editing sequence (MEGA-PRESS) was utilized to accurately detect GABA. MRS data were analyzed and quantified by LCModel software as a linear combination of individual metabolite spectra. We found that, (1) in the thalamus GABA was significantly elevated in welders (p<0.05), and showed the same trend in smelters (p=0.08). In the smelters, the increasing GABA level strongly correlated with the duration of exposure (R=0.867, p<0.01); (2) reduced mI, a glial cell marker, was shown in the thalamus and hippocampus of both welders and smelters (p<0.01 and p<0.05, respectively); (3) decreased GPC was found in the PCC of welders and smelters (p<0.05), and in the hippocampus of welders (p<0.05); (4) Tau, an osmoregulator and modulator of GABA, was significantly decreased in the thalamus of welders (p<0.05), and showed the same trend in smelters (p=0.2); (5) in the PCC the increase in GSH, an antioxidant, directly correlated with the duration of exposure in smelters (R=0.922, p<0.01). Overall, our findings suggest that chronic Mn exposure may lead to disturbance of neurotransmitter and osmoregulation homeostasis as well as damage of glial cells, which may associate with the Mn-induced abnormalities. (Supported by NIH/NIEHS R21 ES-017498).

PS 2141 THE DEVELOPMENT OF A HUMAN MULTI-ROUTE PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR MANGANESE.

M. D. Taylor[1], J. D. Schroeter[2], A. Nong[2], M. Yoon[2], D. C. Dorman[3], M. E. Andersen[2] and H. J. Clewell[2]. *[1]Afton Chemical Corporation, Richmond, VA, [2]The Hamner Institutes for Health Sciences, Research Triangle Park, NC and [3]College of Veterinary Medicine, North Carolina State University, Raleigh, NC.*

Manganese (Mn) is an essential nutrient that can be toxic following overexposure. Increased accumulation of Mn in brain regions such as striatum and globus pallidus is associated with neurotoxicity following high-dose Mn inhalation. A series of physiologically based pharmacokinetic (PBPK) models for ingested and inhaled Mn in rats and nonhuman primates were previously developed. The models contained saturable Mn tissue binding capacities, preferential fluxes of Mn in specific tissues, and homeostatic control processes such as inducible biliary excretion of Mn. In this study, the nonhuman primate model was scaled to humans. Simulation results accurately recapitulated tissue Mn concentration changes following inhalation and radiological tracer Mn exposures in monkeys and humans. These results indicate that the model can accurately simulate the kinetic behavior of Mn and predict exposures that increase Mn concentration in target tissues. A comparison of rat, monkey, and human predictions of target tissue Mn concentrations reveals that all species exhibited similar increases in brain Mn concentrations upon increasing levels of inhalation exposure. Population simulations indicate that changes in globus pallidal Mn concentrations from continuous exposure to air concentrations < 0.01 mg/m^3 Mn as respirable particulates of $MnSO_4$ are small compared with the impact of normal dietary variation. This human Mn PBPK model can become a key component of future human health risk assessments of Mn, allowing the consideration of all exposure routes, natural tissue background levels, and homeostatic controls to explore exposure conditions that lead to increased target tissue levels resulting from Mn overexposure.

PS 2142 DEVELOPMENTAL LEAD EXPOSURE CAUSES TIME POINT SPECIFIC ALTERATIONS IN NERVOUS SYSTEM RELATED GENE AND PROTEIN EXPRESSION LEVELS.

S. Peterson, J. Zhang, G. J. Weber and J. L. Freeman. *Purdue University, West Lafayette, IN.*

Childhood lead (Pb) exposure continues to be an important domestic and international issue. While numerous studies have investigated Pb neurotoxicity, the genetic mechanisms underlying the neurologic dysfunctions associated with environmental Pb exposure are still being elucidated. The goal of this study was to identify novel gene targets and the underlying genetic mechanisms associated with developmental Pb neurotoxicity. First zebrafish embryos were exposed to range of Pb concentrations throughout development to establish relative toxicity. Zebrafish embryos were then exposed immediately following fertilization to a concentration of Pb well below one that induced overt toxicity and global gene expression was analyzed at two developmental time points, 72 hours post fertilization (hpf) and 120 hpf. The list of genes altered due to Pb exposure at the 72 hpf time point was heavily enriched with ontologies associated with neurological development and function, while the later time point showed a lesser number of genes with altered expression and no association with neurological processes. Altered gene expression of a subset of the neurological genes was assessed at additional developmental time points with quantitative PCR. Additionally, translation of the gene expression alterations to the protein level was investigated using western blot analysis. These data further demonstrated developmental time point specific alterations in target gene and protein expression levels supporting the concept that specific endpoints of toxicity can vary by developmental stage and are likely correlated with concurrent

developmental processes. Ongoing work is now exploring the associated morphological and structural changes associated with the identified gene and protein expression alterations.

PS 2143 LOW-LEVEL DEVELOPMENTAL LEAD (PB2+) EXPOSURES ALTERS C-START RESPONSE IN LARVAL ZEBRAFISH.

D. Weber[1] and C. Rice[2]. [1]*Children's Environmental Health Sciences Center, University of Wisconsin Milwaukee, Milwaukee, WI and* [2]*American University, Washington, DC.*

Rationale and Scope: Lead (Pb2+) continues to be a major urban health hazard. Research is required to assess long-term behavioral effects of low-level Pb2+ concentrations and physiological mechanisms that control those behaviors. Experimental Procedures: Newly fertilized zebrafish embryos (<2 hours post fertilization; hpf) were exposed to either: 0.0, 0.01, 0.03, 0.10, 0.30, 1.0 or 10.0 μM lead (as PbCl2)until 24 hpf. Embryo Pb2+ uptake evaluated by ICP-MS after microwave acid digestion. Larvae (168 hpf) were tested for response to a directional, mechanical stimulus. Individual larvae were placed into a chamber (15 mm diam., 3 mm depth). A remotely controlled solenoid arm hit a pin that tapped the chamber. Tap frequency was adjusted to either 1 or 4 hits/sec. Startle response was recorded at 1000 fps by high speed videography and analyzed for latency to reaction (time in msec until initial head movement), maximum turn velocity (Vmax; directional rate of motion of head turn in body lengths/sec) and escape time (time in msec after turn completed and swimming motion ceased). Results: Larvae responded in a dose-dependent pattern for both latency of reaction and Vmax. With increasing exposure concentrations, a larger number of larvae failed to respond to even the initial tap and, for those that did respond, ceased responding earlier than control larvae. These differences were more pronounced at the higher tapping frequency. Conclusions: While it has been known for a long time that Pb2+ is a potent neurotoxicant, the effect of low-level concentrations of Pb2+ during developmentally sensitive periods is only recently being understood. These data suggest that zebrafish are viable models for sensorimotor deficits induced by short-term, low-level developmental Pb2+ exposures. Because our previous studies indicated that no detectable Pb2+ remains in the larvae, it is likely that the behavioral effects observed were due to toxicant-induced embryonic alterations in brain structure and/or function.

PS 2144 NEURODEVELOPMENTAL TOXICITY OF LEAD (PB): A MORPHOLOGIC STUDY IN THE EMBRYONIC ZEBRAFISH BRAIN.

J. Zhang, S. M. Peterson, W. Zheng and J. L. Freeman. *School of Health Sciences, Purdue University, West Lafayette, IN.*

Previous studies have reported that environmental Pb exposure causes neurological dysfunction in children, such as cognitive deficits and attention deficit hyperactivity disorder. However, the specific alterations in neurodevelopmental morphology due to Pb exposure have not yet been thoroughly investigated. Ongoing studies in our laboratory found that global gene expression alterations associated with sublethal developmental Pb exposure at 100 ppb (0.48 μM) were enriched with neurological genes involved in (1) neuronal ontogenesis and (2) synapse formation and function at 72 hours post fertilization (hpf) in the zebrafish. To investigate alterations in neurologic morphology, a top-down approach was initiated to observe general brain morphology, apoptosis of brain cells and axon tract paths in the embryonic zebrafish brain at several time points after exposure to 0, 100 and 10,000 ppb Pb. In addition, the effect of 100 ppb Pb exposure on specific axon tracts was studied in lateral and dorsal view following eye-dissection. Brightfield microscopy showed that, compared with control group, the Pb treatments did not affect the size of the brain or cause any gross malformation. Additionally, there was no increase in apoptosis in brain cells in the Pb treated embryos. Total axon tract staining density, marked by anti-acetylated tubulin antibody staining, in multiple regions of the brain in dorsal view, also showed no differences between the control and Pb treatments. In addition, no significant changes were observed in the general pattern of axon tracts, including the anterior commissure, post-optic commissure, dorsal rostral neuronal cluster or posterior commissure, at 24 hpf following 100 ppb Pb exposure. From the endpoints analyzed to date, there is a lack of morphological alterations in the embryonic zebrafish brain induced by Pb exposure at the tested doses. More detailed experiments are now being conducted at additional developmental stages to investigate subtle alterations in neuron structure and density. (Supported by NIH/NIEHS R21-ES017055)

PS 2145 EFFECTS OF DEVELOPMENTAL LEAD EXPOSURE ON GENE EXPRESSION IN THE HIPPOCAMPUS: GENE-ENVIRONMENT INTERACTIONS.

W. Mettil, D. Anderson, L. Walinchus, R. Schray and J. Schneider. *Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA.*

Sex, rearing environment and dose of developmental lead (Pb) exposure may interact in complex ways and act as modifiers of the association between Pb exposure and behavioral outcome. However, the influences of Pb, sex and environment on genes important for normal development and function of the hippocampus have not been studied. Thus, the present study was performed to examine the influence of these factors on select hippocampal gene expression profiles. Dams were fed Pb-containing food for 10 days prior to breeding and remained on the same diet through weaning. Pups were weaned onto control chow and either housed 3 to a cage with no added toys (social/impoverished) or 6 to a larger cage with added toys that were changed every 3rd day (enriched). At day 55, animals were euthanized and the hippocampus rapidly removed and frozen. RNA was extracted and reverse transcribed. Gene expression was analyzed using a Roche LightCycler 480 Real-time PCR System II. Genes related to major neurotrophic factors (ex., BDNF, bFGF, NGF, NT3) were analyzed along with genes coding NMDA receptor subunits (ex., NR1, NR2A, NR2B, NR2C, NR3A, NR3B), metabotropic glutamate receptors (ex., mGluR3, mGluR4, mGluR5), genes related to synaptic plasticity, learning and memory (ex., CaMKII a and b, RIM1, synaptophysin, spinophilin), and miscellaneous genes involved in gene transcription and regulation and glial function (ex., ZFP3, DNMT1, AMOG, DRG1, CNTN2). Many of these genes were differentially expressed in control male and female brain. Complex interactions between level of Pb exposure, gender and environment were observed. Expression of several genes was significantly altered at the lowest level of exposure and further increases in Pb exposure did not significantly modify the effect. These data suggest that developmental Pb exposure affects the expression of a wide range of genes in the hippocampus and that these effects can occur at low exposure levels, are gender-specific and may be modified by rearing environment. Supported by ES015295.

PS 2146 LEAD DIFFERENTIALLY AFFECTS GENE EXPRESSION PROFILES IN HIPPOCAMPUS AND FRONTAL CORTEX.

J. Schneider, W. Mettil, D. Anderson, R. Shray and L. Walinchus. *Thomas Jefferson University, Philadelphia, PA.*

Despite attempts to reduce environmental sources of exposure, childhood lead (Pb) poisoning remains a serious public health problem. Children exposed to Pb have a variety of learning, memory, attention and executive function problems reflecting effects of Pb on a variety of brain regions including the hippocampus and frontal cortex. Although early childhood exposures are more common, exposure to Pb during late childhood and adolescence can also have detrimental consequences for brain maturation and adult cognitive/behavioral functions. The present study examined the effects of a brief postweaning exposure to Pb on gene expression profiles in the hippocampus and frontal cortex. Male Long-Evans rats, housed 4 per standard cage, were weaned onto diets containing Pb acetate (180ppm, 375ppm or 1,500 ppm) or control diet without Pb for 30 days. Animals were euthanized at day 55 and hippocampus and frontal cortex were removed, RNA extracted, and reverse transcribed. Gene expression was analyzed using a Roche LightCycler 480 Real-time PCR System II. Genes related to major neurotrophic factors (ex., BDNF, bFGF, NGF, NT3) were analyzed along with genes coding NMDA receptor subunits (ex., NR1, NR2A, NR2B, NR2C, NR3A, NR3B), metabotropic glutamate receptors (ex., mGluR3, mGluR4, mGluR5, mGluR8), genes related to synaptic plasticity, learning and memory (ex., CaMKIIa and CaMKIIb, RIM1, synaptophysin, spinophilin), and genes involved in gene transcription, regulation, and glial function (ex., ZFP3, MeCP2, DNMT1, AMOG, CNTN2). Many of these genes were differentially expressed in the 2 structures in control animals and Pb affected expression of most genes differently in cortex vs. hippocampus. Expression of a number of genes was significantly altered at the lowest level of exposure while other genes had a more complex dose-response profile. These data suggest that even a brief postweaning lead exposure can significantly affect expression of diverse genes in the hippocampus and frontal cortex. Supported by ES015295.

PS 2147 CATALASE MANIPULATIONS MODIFY VOLUNTARY ETHANOL INTAKE IN DEVELOPMENTALLY LOW-LEVEL LEAD EXPOSED RATS.

M. Virgolini, M. S. Mattalloni and L. M. Cancela. *IFEC CONICET Department . de Farmacologia., Fac. de Ciencias Quimicas. Universidad Nacional de Cordoba, Cordoba, Argentina.*

Lead (Pb) is a developmental neurotoxicant that modify several responses to drugs of abuse. We have demonstrated that low-level Pb-exposed rats showed increased ethanol (ET) consumption in a limited free-choice paradigm compared to their control counterparts. The current study sought to investigate the role of catalase (CAT) on ET intake, being CAT an enzyme implicated in both, brain ethanol metabolism, and Pb-induced anti-oxidant defense system. We postulate that concomitant exposure to Pb and ET would stimulate CAT activity leading to the higher ET intake observed in developmentally Pb-exposed animals. Thirty-five day old animals exposed to 220 ppm Pb during gestation and lactation were evaluated in their voluntary ethanol intake during 2 h for 28 days according to the following scheme: days 1-4: ET 2%; days 5-8: ET 4%; days 9-12: ET 6%; days 13-16: ET 8%; days 17-20: ET 10%. After stable 10% ET consumption was achieved, all rats were injected with: 1) vehicle (saline); 2) an inhibitor of CAT activity: AT, 3-amino 1,2,4 triazole (0.25 mg/kg i.p.) 5 h before the onset of the daily ET choice session (days 21-28), or 3) an activator of CAT activity: 3-NPA, 3 nitropropionic acid (10, 20, and 30 mg/kg s.c.) 90 min before the corresponding ET choice session (days 25-28). Immediately after the last free-choice session, all rats were sacrificed and several brain regions harvested to measure brain CAT activity. Blood was also collected to measure CAT activity, Pb, and ET levels. A 35-day-old and a 70-day-old group that have not consumed ethanol were included. We demonstrated that pretreatment with AT blunted, whereas 3NPA (20 mg/kg) further increased the higher voluntary ET intake previously observed in Pb-exposed rats. Corresponding changes were observed in blood and brain CAT activity. These results emphasize the importance of CAT as an antioxidant enzyme and a key component of brain ET metabolism, ascribing a critical role for acetaldehyde in Pb-induced ET reinforcing effects.

PS 2148 WATERBORNE MANGANESE EXPOSURE ALTERS STEREOTYPIC BEHAVIORS AND PLASMA METABOLITES IN DEVELOPING RATS.

S. C. Fordahl[1], P. Cooney[1], Y. Qiu[2], G. Xie[2], W. Jia[2] and K. Erikson[1]. *[1]Nutrition, University of North Carolina at Greensboro, Greensboro, NC and [2]Nutrition, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC.*

Symptoms of manganese (Mn) neurotoxicity appear as Mn accumulates in the basal ganglia (notably the striatum and globus pallidus (GP)), but to date no reliable biomarkers have been identified to correspond with early Mn neurotoxicity. The purpose of this study was to identify plasma metabolites that correlate with GP and striatal Mn accumulation and the onset of stereotypic behavioral changes. Twenty-one day old rats exposed to 1g Mn/L (as MnCl2) drinking water for six weeks were monitored for stereotypic behaviors over a 24hr period using video surveillance in their home-cage environment. This surveillance protocol is advantageous to accurately capture behavior abnormalities that persist under "normal living" conditions. At the end of the study, plasma, GP and striatal samples were analyzed for Mn, iron, and metabolomic data. Pearson's correlations were made between these data and total distance traveled (TDT), turn, sniff, rear, and grooming resulting in several significant relationships linking Mn to these behaviors. Plasma Mn was significantly correlated with GP Mn (r=0.455, p=0.025), TDT (r=0.459, p=0.024), and repetitive turning (r=0.589, p=0.002), but not striatal Mn. Striatal Mn was only linked to the plasma iron:Mn ratio (r=-0.439, p=0.032), which also was a stronger predictor of TDT and turn behavior. Similarly, TDT was significantly associated with GP and striatal Mn, but turning was only linked to GP Mn. Significant associations between, GP Mn, TDT and repetitive turning also emerged with several plasma metabolites; most notably, homogentisic acid (r=0.603, r=0.420, and r=0.598, respectively), an indicator of tyrosine metabolism. These data reveal that relationships between plasma Mn, GP Mn, plasma metabolites and altered stereotypic behaviors can lead to the identification of reliable biomarkers of Mn neurotoxicity.
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PS 2149 PRE-WEANING MN EXPOSURE LEADS TO PROLONGED ASTROCYTE ACTIVATION AND LASTING EFFECTS ON THE DOPAMINERGIC SYSTEM IN ADULT RATS.

C. Kern and D. R. Smith. *Environmental Toxicology, University of California, Santa Cruz, CA.*

Little is known about the effects of manganese (Mn) exposure over neurodevelopment and whether these early insults result in effects lasting into adulthood. To determine if early Mn exposure produces lasting neurobehavioral and neurochemical effects, we treated neonate rats with oral Mn (0, 25, or 50 mg Mn/kg/d over PND 1–21) and evaluated 1) behavioral performance in the open arena in the absence (PND 97) and presence (PND 98) of a d-amphetamine challenge, 2) brain dopamine D1 and D2-like receptors and dopamine transporter densities in the prefrontal cortex, striatum, and nucleus accumbens (PND 107), and 3) astrocyte marker glial fibrillary acidic protein (GFAP) levels in these same brain regions (PND 24 and 107). We found that pre-weaning Mn exposure did not alter locomotor activity or behavior disinhibition in adult rats, though Mn-exposed animals did exhibit an enhanced locomotor response to d-amphetamine challenge. Pre-weaning Mn exposure led to increased D1 and D2 receptor levels in the nucleus accumbens and prefrontal cortex, respectively, compared to controls. We also found increased GFAP expression in the prefrontal cortex in Mn-exposed PND 24 weanlings, and increased GFAP levels in prefrontal cortex, medial striatum and nucleus accumbens of adult (PND 107) rats exposed to pre-weaning Mn, indicating an effect of Mn exposure on astrogliosis that persisted and/or progressed to other brain regions in adult animals. These data show that pre-weaning Mn exposure leads to lasting molecular and functional impacts in multiple brain regions of adult animals, long after brain Mn levels returned to normal.

PS 2150 EFFECTS OF PERINATAL LEAD EXPOSURE ON LEARNING AND MEMORY ARE MODIFIED BY SEX AND REARING CONDITION.

K. Pothakos, D. Anderson, R. Schray, L. Walinchus and J. Schneider. *Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA.*

Recent clinical data suggest that gender and environment may act as modifiers of the association between lead (Pb) exposure and neuropsychological outcome. However, the influences and potential interactions between these factors on behavioral outcome from developmental Pb exposure has received little attention experimentally. Thus, the present study was performed to examine the influence of these factors on behavioral outcome of rats exposed to different levels of Pb during development. Dams were fed Pb-containing food for 10 days prior to breeding and remained on the same diet through weaning. Pups were weaned onto control food without Pb. At weaning, animals were either housed 3 to a cage with no added toys (social/impoverished) or 6 to a larger cage with added toys that were changed every 3rd day (enriched). At day 55, males and females were assessed for spatial learning and reference memory in a Morris water maze. There was a significant effect of environment on learning in both males and females with control enriched animals performing significantly better than control impoverished animals. In females, enrichment overcame the detrimental effects of Pb on learning at all but the highest level of Pb exposure. In males, enrichment had no effect at the lowest level of exposure but counteracted effects of Pb at higher exposure levels. Enriched females at all but the highest level of Pb exposure performed better on probe tests (eg. percent of time in platform/target area) than matched impoverished animals. In males, enrichment only had an effect on probe performance in the highest Pb exposure group. These data suggest that there are complex interactions between gender, rearing environment, and developmental Pb exposure on behavioral outcomes and that these relationships need to be studied in greater detail spanning different exposure paradigms and cognitive functions. Supported by ES015295.

PS 2151 A MOUSE MODEL OF Pb-STRESS INTERACTIONS AT BLOOD Pb < 10 μg/dl.

K. Merchant-Borna, S. Liu, D. Weston and D. A. Cory-Slechta. *Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY.*

Lead (Pb) exposure and psychological stress are co-occurring risk factors that preferentially target low SES communities. Both act on the brain mesocorticolimbic dopamine system and the hypothalamic pituitary-adrenal (HPA) axis. Stress hormones impact brain dopamine systems and can thereby influence associated behaviors. Previous studies have demonstrated persistent interactions in rats of both maternal and continuous Pb exposure with prenatal stress in offspring of both genders. The current study extended these observations to a mouse (C57BL/6J) model to

further determine neuroendocrine and neurochemical mechanisms of Pb-stress interaction effects on behavior. Similar behavioral, corticosterone (CORT) and neurochemical outcomes as in prior studies were used here to facilitate cross-species comparisons. Offspring were chronically exposed to 0 or 100 ppm Pb in drinking water that had begun in dams 2 mos prior to breeding, to prenatal restraint stress (PS), or to both. Behavior was evaluated using a fixed-interval (FI) schedule of food reinforcement, a baseline with demonstrated sensitivity to Pb. Offspring blood-Pb values averaged <0.1 and 7.4 μg/dl, respectively. Males maintained higher FI overall response rates (OR) than females. In males, combined Pb and PS preferentially increased FI OR by approximately 33% relative to controls and which were positively related to CORT levels. The increased FI OR appeared to derive primarily from increased run rates. Females exhibited a similar but not-significant profile of FI behavioral changes. CORT levels measured post-FI testing (time1) and 1 mo later (time2) showed time-related increases in both genders. PS decreased CORT levels by 40-65% in females at time1, but no group differences were evident at time2. Males showed no treatment-related differences at either time point. The effects of combined Pb and stress among males in the absence of effects of either variable alone demonstrate the importance of considering multiple risk factors simultaneously and differentially by gender. ES012712, ES07026, ES01247

R 2152 'OMICS IN TOXIC TORT.

A. J. Harris. *CTEH, North Little Rock, AR.*

In the past few years, there has been a growing influx of 'omics data into the courtroom. Transcriptomic and proteomic data have already been submitted in toxic tort litigation as evidence of exposure to a chemical or toxic effect due to chemical exposure, as have data regarding the genetic polymorphisms of plaintiffs alleging toxic effects. Submission of 'omics data will likely increase exponentially over the next decade, however, there has been little discussion in the scientific community about how appropriate these data currently are in proving exposure and effect in any individual or group of individuals. Issues regarding specificity, interindividual and intraindividual variation, and validation are addressed in the scientific community as the data are projected for use in making regulatory decisions, but there is a serious gap in gauging the sometimes limited datasets submitted as evidence on which to base causal decisions in a court of law. We will introduces topics related to 'omics in toxic tort to open a dialogue among toxicologists interested in the transition of these data from research tools to causative tools in litigation.

IS 2153 CURRENT AND CHANGING PERSPECTIVES ON MYCOTOXINS AND THEIR POTENTIAL HEALTH RISKS WORLDWIDE.

K. A. Voss[1], M. Bolger[2], S. H. Henry[2], J. G. Adams[3] and F. Wu[4]. [1]*Toxicology & Mycotoxin Research Unit, USDA-ARS, Athens, GA,* [2]*Center for Food Safety and Applied Nutrition, U.S. FDA, College Park, MD,* [3]*Almond Board of California, Modesto, CA and* [4]*Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA.*

Mycotoxins are ubiquitous contaminants of cereals and other commodities. Interventions at the agricultural, commodity processing, or food preparation stages of the field to plate sequence have significantly contributed to reducing human exposures. However, establishment of tolerable intakes and regulatory policies limiting the levels of aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, and other mycotoxins known or suspected to cause human disease continues as a first line of defense for protecting consumer health. Contrasting approaches to safety and risk assessment can lead to the establishment of different tolerable intakes or regulatory standards as, for example, between the U.S. and the E.U. Improved commodity sampling and monitoring procedures and additional toxicology data reduce uncertainty in risk assessment and allow refinement of regulations for mycotoxins, as illustrated by changes in limits for aflatoxins in tree nuts which have facilitated international trade without compromising public health. However, regulatory approaches are not globally consistent and may not effectively protect public health if enforcement is less stringent. Inconsistent standards can in some cases lead to exportation of the best quality commodities. Therefore, implementation of multifaceted bottom up approaches involving better pre-harvest and post-harvest practices, improved nutrition, and clinical interventions is also needed to effectively reduce exposures to aflatoxins and other mycotoxins. Recent research increases our understanding of how mycotoxins interact with commodity and food matrix constituents, describes improved biomarkers for exposure assessments, and provides new findings on the toxicology and mechanisms of action that are essential for both regulatory and bottom up approaches to reduce mycotoxin exposure and maintain safe food supplies worldwide.

PS 2154 TERPENE EFFECTS ON PENETRATION OF NANOPARTICLES IN HUMAN SKIN.

N. A. Monteiro-Riviere[1], A. O. Inman[1], D. Erdmann[2], X. Xia[1] and J. E. Riviere[1]. [1]*Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC and* [2]*Duke University Medical Center, Duke University, Durham, NC.*

Solvents and vehicles can alter the barrier properties of skin after occupational or accidental spillage. Skin is the largest organ of the body and a primary route of exposure to commercialized nanomaterials. Quantum dot (QD) nanoparticles have great potential for use in the electronics industry, drug delivery, diagnostic, and imaging agents in biomedicine due to their superior photoemission and photostability characteristics. Spherical QD-565 have a Cd/Se core and ZnS shell. QD-565 with polyethylene glycol amine (PEG-NH_2; cationic) and carboxylic acid (COOH; anionic) surface coatings and hydrodynamic diameters of 15nm and 14nm, respectively, were used. Terpenes are highly lipophilic aliphatic compounds in essential oils that are generally regarded as safe. Human skin was occluded and perfused for 1h in flow-through diffusion cells with no pretreatment or pretreatments of menthol, eucalyptol, limonene, or ethanol vehicle at 225.6μl/cm^2. QD-565 were topically applied to human skin at a concentration of 62.5pmoles/cm^2 and perfused occluded for 8h. Perfusate samples were collected hourly and analyzed for fluorescence. At termination, skin was harvested and frozen for analysis with laser scanning confocal microscopy (LSCM) and fixed for transmission electron microscopy (TEM). Fluorescence and LSCM of both QD surface coatings and sizes revealed minimal QD penetration through the stratum corneum (SC) layers. By TEM, QD were located deeper into the SC with terpene treatments compared to control, but QD still remained within the nonviable SC layers. These studies showed that terpenes caused only a slight enhancement of QD penetration into the SC but no absorption occurred through skin.

PS 2155 SKIN PH EFFECTS THE PENETRATION OF QUANTUM DOT NANOPARTICLES IN PORCINE SKIN.

A. O. Inman, N. A. Monteiro-Riviere and J. E. Riviere. *Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC.*

Skin is the largest organ of the body and a primary route of exposure to nanomaterials. Quantum dot (QD) nanoparticles are utilized in medicine for diagnostics, imaging, and fluorescent tracking of cells and drug delivery due to their intense stable fluorescence and flexible surface chemistry. Commercial nanoparticles are stored in different vehicle pH to maintain stability and to prevent agglomeration. However, certain pH of buffers may alter the barrier properties of the skin and effect the nanoparticle penetration. Commercial spherical QD-565 having a Cd/Se core and ZnS shell with a polyethylene glycol amine (PEG-NH_2; cationic) surface coating with a hydrodynamic diameter of 15nm in borate buffer was used. Skin on the back of the pig was predosed in vivo for 1h with borate buffer at pH 7.8, pH 8.3, pH 9.0, or pH 9.7 and transepidermal water loss (TEWL) measured relative to normal skin. While TEWL measurements increased slightly after pretreatment with all four of the borate buffers, no skin erythema or edema was noted in the sites at any pH. The pig was euthanized and the skin dermatomed at 400μm and placed in flow-through diffusion cells. After the skin was equilibrated, QD-565-PEG-NH_2 were topically applied to the skin at 62.5pmoles/cm^2 and perfused for 8h. Perfusate samples were collected hourly and analyzed for fluorescence. At termination, the skin was harvested and half frozen for analysis with laser scanning confocal microscopy (LSCM) and half fixed for light microscopy (LM). LM of skin pretreated with borate buffer at pH 7.8 revealed a slight increase in epidermal alterations with mild subepidermal vacuolization, while at pH 8.3 focal apoptic cells were present and at pH 9.7 a slight increase in acanthosis was noted. LSCM found no evidence of a pH-induced increase in QD penetration, although there was a statistically significant ($p<0.05$) increase in perfusate fluorescence relative to the 0h perfusate samples. This study shows that pH has minimal effect on the penetration of commercial QD through pig skin.

PS 2156 INFORMING SELECTION OF NANOMATERIAL CONCENTRATIONS FOR TOXCAST *IN VITRO* TESTING USING THE MULTIPLE-PATH PARTICLE DOSIMETRY MODEL.

S. Gangwal[1], J. S. Brown[2], A. Wang[1], K. A. Houck[1], D. J. Dix[1], R. J. Kavlock[1] and E. A. Cohen Hubal[1]. [1]*National Center for Computational Toxicology (NCCT), U.S. EPA, Research Triangle Park, NC and* [2]*National Center for Environmental Assessment (NCEA), U.S. EPA, Research Triangle Park, NC .*

Currently, little justification is provided for nanomaterial testing concentrations in *in vitro* assays. The *in vitro* concentrations typically used may be higher than those experienced by exposed humans. Selection of concentration levels for hazard evalu-

ation based on real-world exposure scenarios is desirable. Nanomaterial concentrations in air (particle mass or count per air volume) are being measured in manufacturing and R&D lab settings. We reviewed nanomaterial levels reported across facility types for different nanomaterial classes. Using particle number concentration data from these studies, we calculated nanomaterial mass retained in the tracho-bronchial and alveolar regions of the human lung using the open-source Multiple-Path Particle Dosimetry (MPPD) model. These estimates of inhalation dosimetry were performed for carbon nanotubes (CNTs), titanium dioxide (TiO_2) and silver nanoparticles. The key model input parameters that affect the alveolar mass retained after 24 hours of nanoparticle exposure were particle size and size geometric standard deviation, aspect ratio, breathing conditions (resting, light or heavy exercise), and aerosol concentration. These key parameters were varied to further calculate alveolar mass retained per alveolar surface area (μg/cm^2) for different particle sizes (ranging from 5 to 100 nm), aerosol concentrations (0.1 and 1 mg/m^3), and exposure times (24 hours and a full working lifetime of 45 years at 8 hours per day, 5 days per week of aerosol inhalation). The alveolar mass retained per surface area for silver and TiO_2 nanoparticles for *a full working lifetime* exposure duration was similar to the high-end concentrations (~ 100-200 μg/mL) typical of *in vitro* testing for silver and TiO_2 nanoparticles. The amount retained per surface area after 24 hours of exposure equated to approximately 0.5 μg/mL. *This abstract may not necessarily reflect U.S. EPA policy.*

PS 2157 NANOTOXICOLOGY – QUO VADIS?

H. F. Krug. *Materials Meet Life, EMPA - Materials & Technology, St. Gallen, Switzerland.* Sponsor: B. Fadeel.

Nanosafety considerations become a substantial part of many funding programs, international conferences, regulatory activities worlwide and many international working groups are taking care of this topic. Since the term "Nanotoxicology" appeared the first time within a title of a publication [1] confusion about its implementation and the objectives starts. One year later, the relationship to the former research on ultrafine particles has been highlighted [2] and the "new discipline" started its success story. Whereas the number of publications regarding nanotoxicological issues from that time increases dramatically each year (from around 150 in 2004 to nearly 1300 in 2009) clarity about possible risks dropped. Besides the fact that more and more products contain nanoparticles (NP) and thus have to be tested to be safe for the customer there is no general opinion how to approach this target. It has been shown that many of the used methods are not adapted for NP and thus results are often false-positive or false-negative. Not only we could demonstrate the interference of NP with the assay systems which often is neglected [3], solvent problems have not been addressed and the appropriate controls are missing as well as the characterization of the materials tested. Taking all these critical points into account especially nanotoxicology has to establish a new strategy in the future as has been postulated last year [3] on the basis of important recommendations [4]. [1] Donaldson K, Stone V, Tran CL, Kreyling WG, & Borm PJ (2004) Nanotoxicology. Occup Environ Med 61: 727-728; [2: Oberdörster G, Oberdörster E, & Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113: 823-839; [3] Wörle-Knirsch JM, Pulskamp K, & Krug HF (2006) Oops they did it again! Carbon nanotubes hoax scientists in viability assays. Nano Lett 6: 1261-1268; [4] Hartung T (2009) Toxicology for the twenty-first century. Nature 460: 208-212; [5] Krug HF & Wick P (2010) Nanotoxicology – an interdisciplinary challenge. Angew Chem Int Ed, accepted

PS 2158 A NOVEL COMPREHENSIVE EVALUATION PLATFORM TO ASSESS NANOPARTICLE TOXICITY *IN VITRO.*

C. Hirsch[1], F. Wessling[1], K. Fischer[1], M. Roesslein[1], P. Wick[1], H. Hofmann[2] and H. F. Krug[1]. [1]*Materials-Biology Interactions, Empa, Swiss Federal Laboratories for Materials Science and Technology, St. Gallen, Switzerland and* [2]*Powder Technology Laboratory, EPFL, Lausanne, Switzerland.* Sponsor: B. Fadeel.

The unique properties of engineered nanomaterials (ENM) render them suitable for various applications. However, even though studies on biological effects of ENM are available standardized and validated test systems are still missing. Furthermore, inappropriate ways of suspending ENM and their interference with different assays frequently lead to false results. Comparing data to profoundly estimate nano-related toxicity is thus virtually impossible. Hence, harmonized, robust and comprehensively validated tools to assess toxicological effects of ENM are urgently needed. Our novel in vitro evaluation system addresses four key aspects of cytotoxicity: viability, inflammation, genotoxicity and oxidative stress. Special emphasis is laid on careful reassessment and validation of the tests and a thorough characterization of the ENM. Interlaboratory comparisons will subsequently verify the reliability, reproducibility and robustness of the testing platform.

Cell viability was assessed using AnnexinV/Propidium Iodide double-labeling of lung epithelial cells treated with increasing concentrations of ZnO nanoparticles (NP). We find a dose-dependent increase in the number of late apoptotic/necrotic cells. To distinguish effects caused by Zn-ions from NP-related ones we compared dose-response curves of ZnCl2 and NP treated cells. Preliminary results indicate that ZnO NP toxicity is at least to some extend caused by dissociating Zn-ions. Genotoxic effects of different ENM were analyzed by the Comet and Micronucleus assay. At higher concentrations the tested ENM interfere with the system by either quenching or enhancing the fluorescence signal.
We conclude that it is crucial to control for interference of ENM with the assay system as well as for nano-specific effects in comparison to bulk material caused effects. Accordingly, critical concentrations of ENM mustn't be exceeded and additional tests that circumvent the mentioned problems are needed.

PS 2159 PROTEOME PROFILING OF BEAS-2B CELLS TREATED WITH TITANIUM DIOXIDE REVEALS POTENTIAL TOXICITY OF AND DETOXIFICATION PATHWAYS FOR NANOMATERIALS.

M. E. Bruno, W. Winnik, K. Wallace, R. Prasad and Y. Ge. *U.S. EPA, Research Triangle Park, NC.* Sponsor: E. Kenyon.

Oxidative stress is known to play important roles in nanomaterial-induced toxicities. However, the proteins and signaling pathways associated with nanomaterial-mediated oxidative stress and toxicity are largely unknown. To identify oxidative stress-responding toxicity pathways and networks that are associated with exposure to nanomaterials, an integrated redox proteomic study was conducted using human bronchial epithelial cells (BEAS-2B) and titanium dioxide. Utilizing two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS), we identified 46 proteins that were altered at protein expression levels. The protein changes detected by 2-DE/MS were verified by functional protein assays. These identified proteins include some key proteins involved in cellular stress response, metabolism, adhesion, cytoskeletal dynamics, cell growth, cell death, and cell signaling. The differentially expressed proteins were mapped using Ingenuity Pathway Analyses™ (IPA) canonical pathways and IPA tox lists, and these proteins were found to be involved in 20 proteomic pathways/lists. The protein-generated IPA canonical pathways and IPA tox lists were compared to signaling pathways generated from genomic analyses of BEAS-2B cells treated with titanium dioxide. There was a significant overlap in the specific pathways and lists generated from the proteomic and the genomic data. In addition, we also analyzed the phosphorylation profiles of protein kinases in titanium dioxide-treated BEAS-2B cells for a better understanding of upstream signaling pathways in response to the titanium dioxide treatment and the induced oxidative stress. In summary, the present study provides the first protein interacting network maps and novel insights into the biological responses and potential toxicity and detoxification pathways of titanium dioxide. This abstract does not necessarily reflect EPA policy.

PS 2160 SWCNT EXPOSURE OF ALVEOLAR EPITHELIAL CELLS AND MACROPHAGES INDUCED OPN AND TGF-β1 RESPONSE.

M. J. Waltz[1, 2], A. R. Murray[1, 2], E. Kisin[2] and A. A. Shvedova[1, 2]. [1]*West Virginia University, Morgantown, WV and* [2]*NIOSH, Morgantown, WV.*

Exposure of the lung to carbonaceous nanoparticles: single walled carbon nanotubes (SWCNT) leads to the development of pulmonary fibrosis (PF) and induction of a network of pro-fibrotic/pro-inflammatory cytokines including TGF-β1 and osteopontin (OPN). TGF-β1 is involved in fibrotic remodeling including fibroblast differentiation and enhanced deposition of collagen in the extracellular matrix. As part of the inflammatory cascade, OPN acts as a chemoattractant to guide polymorphonuclear neutrophils (PMN) and macrophages (MΦ) to the area of insult/injury and is also involved in collagen deposition. Potential interactions between OPN and TGF-β1 have not been investigated even though both cytokines have been shown to be upregulated in response to SWCNT. We hypothesize that OPN increase in response to SWCNT potentiate TGF-β1 production in lung cells. To test our hypothesis two cell types, alveolar epithelial cells (MLE-15) and RAW 264.7 MΦ were treated with SWCNT (6 μg/cm^2 – 48 μg/cm^2, for 24 hours). Exposure of the cells to SWCNT resulted in significantly enhanced OPN released found in the incubation medium (39.1% for MLE-15 and 38.6% for RAW 264.7 MΦ vs. respective controls). Using an OPN antibody we demonstrated an inhibition of OPN production in response to SWCNT. Further studies are needed to evaluate interplay between OPN and TGF-β1 and their role in SWCNT induced pulmonary fibrosis.

PS 2161 MEMBRANE-MEDIATED MODES OF ENTRY OF C_{60} INTO CULTURED IMMORTALIZED MACROPHAGES.

K. A. Russ[1], I. C. Speirs[1], P. Elvati[2], J. A. Fernandez[1], A. Violi[2] and M. A. Philbert[1]. [1]*Department of EHS, Toxicology, University of Michigan, Ann Arbor, MI and* [2]*Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI.*

Hydrophobic fullerenes such as C_{60} have been studied in several cell types and are cytotoxic. This study examines the interactions of C_{60} with cultured RAW 264.7 immortalized macrophages and its effects on inflammatory potential and cell death pathways. Cells were exposed to five concentrations of fullerenes (0.1, 0.2, 0.76, 1, 4 μg/mL) for 24 hours and were monitored for viability, alterations in cell proliferation, and activation of caspases. An inverted "U-shaped" dose response for viability and activation of caspases was observed, as was a "U-shaped" curve for cell proliferation. Cells exposed to 0.1 μg/mL C_{60} for 24 hours showed decreased global expression of cytokines/chemokines. 24 hour exposure to 4 μg/mL C_{60} caused a global increase in cytokine/chemokine gene expression. Examination of cells by transmission electron microscopy (0.1 and 0.36 μg/mL Tb-endohedral C_{60}) revealed the presence of at least three different pathways by which these nanoscale structures gain access to the cell. There was clear morphological evidence of endocytosis (a mechanism well described by others), diffusion into the membrane, and an unknown pathway unbound by lipid membranes leading to deposition in the nucleus. Based on the data, we hypothesize that the increased metabolic activity is due to the increase in energy required to endocytose or exocytose particles, and the increase in cell growth could be attributed to particles interacting with growth receptors in the cell membrane. Predictive computational models of the interaction(s) between C_{60} and lipid bilayers confirm the most favored disposition of C_{60}. Additional investigation is required to determine the biological and toxicological significance of each pathway and their role in the ultimate fate of the cell. This work was supported by in part by 2R01 ES008846 (MAP), NCI R33 CA125297 (MAP), NIEHS Training Grant T32-ES007062-26 and NSF-CAREER Award CBET 0644639 (AV).

PS 2162 *IN VITRO* TESTING STRATEGIES FOR ASSESSING PARTICLE-INDUCED GENETIC DAMAGE USING ALVEOLAR CELL LINES.

K. P. Glover, A. Myhre, M. Laskowski, K. L. Reed, E. Donner and D. B. Warheit. *DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE.*

New *in vitro* testing strategies are necessary to better simulate *in vivo* exposure conditions, and there might be a need to refine and develop endpoints specific to particle toxicity. Results are shown from studies with alveolar cell lines, such as macrophages, that can retain characteristics of cells that are exposed through *in vivo* inhalation exposure and therefore more appropriate for *in vitro* testing. The purpose of this experiment was to analyze 5 different particle types with different levels of toxicity (carbonyl iron (CI), crystalline silica (CS), amorphous silica (AS) and fine and nanosized zinc oxide (ZnOf and ZnOn)) for their genotoxic potential in two cell lines; the rat alveolar epithelial cell line, L2, and the rat alveolar macrophage cell line, NR8383. The cells were exposed to CI, AS and CS at 10 – 100 μg/cm^2. However, ZnO was 10 fold more toxic than the other particles and was used at 1 – 10 μg/cm^2. Particle induced cytotoxicity was measured through the trypan blue exclusion method and the MTT assay. Genetic damage was assessed using the *In vitro* MicroFlow® Micronucleus assay kit (Litron Laboratories, Rochester, NY) and the alkaline comet assay. In the *in vitro* micronucleus assay, a positive response was observed for CI and both ZnO particles (≥ 2 fold increase relative to the negative control) at particle exposure conditions that induce very high toxicity (< 50% survival). According to the comet assay both L2 and NR8383 cells treated with CI had significantly increased mean tail moments compared to the control. However, this effect was not dose dependent and the highest response was not observed at the highest dose level tested, 100 μg/cm^2. More research efforts need to focus on how to accurately determine potential particle induced genetic damage using modified assay conditions that account for the unique properties of particles in the traditionally validated assays.

PS 2163 DEVELOPING AN *IN VITRO* MODEL OF THE ALVEOLO-CAPILLARY BARRIER TO STUDY TOXICITY AND TRANSLOCATION OF NANOPARTICLES.

K. Luyts, B. Nemery and P. H. Hoet. *Research Unit for Lung Toxicology, K.U.Leuven, Leuven, Belgium.*

Due to their small size, nanoparticles (NP) can penetrate deep into the lungs and reach the alveoli where they can cross the alveolar barrier and reach the pulmonary vessels. Therefore, an in vitro model of the alveolo-capillary barrier might be a useful tool to study the effects and translocation of NP. The human pulmonary epithelial 16HBE14o- cell line (epi) and human endothelial EAHY926 cell line (endo) were seeded on opposite sides of a permeable membrane support creating a bilayer. Two conformations were tested: 16HBE14o- on apical side/EAHY926 on basolateral side (epi/endo) and the other way around (endo/epi). Permeable supports with different pore size were used: 0.4 and 3 μm. Transepithelial electrical resistance (TEER) measurements were performed daily to check cell layer integrity and to compare with monocultures (epi or endo on apical side). Using 0.4 μm pore supports, the TEER values of the epi/endo bilayer were significantly higher compared to the monolayers starting from three days after seeding. From five days after seeding the TEER values of the endo/epi model were significantly higher compared to both monolayers. Both conformations reached their maximum TEER five days after seeding: 1596 ± 106.8 Ω.cm2 (epi/endo) and 1559 ± 311.1 Ω.cm2 (endo/epi) compared to 814.8 ± 285.8 Ω.cm2 (epi) and 5.198 ± 0.3160 Ω.cm2 (endo). Using 3 μm pore supports, maximum TEER values were lower (375.7 ± 140.7 Ω.cm2 epi/endo; 571.9 ± 109.7 Ω.cm2 endo/epi) compared to those obtained with 0.4 μm pore supports and obtained six days after seeding. The results indicate a tight epithelial barrier and the presence of endothelial cells in the model accounts for significantly higher TEER values compared to the epi monolayer. This makes the model suitable to study the effects of NP (and other substances) on the alveolo-capillary barrier (inflammation, oxidative stress) in two different conformations. Moreover, 3 μm pore supports allow the study of interactions between epithelial and endothelial cells as well as particle translocation. EU-FP7: ENPRA; FWO:F.OS47.08

PS 2164 CYTOTOXICITY AND GENOTOXICITY OF ACUTE AND CHRONIC EXPOSURE TO SILVER NANOPARTICLES IN HUMAN LUNG CELLS.

H. Xie[1, 2, 3], D. McGovern[1, 2], A. Sample[1, 2], S. Abramson[1, 2], A. Perez[1, 2], M. D. Mason[4], G. Craig[4] and J. P. Wise[1, 2, 3]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME,* [2]*Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME,* [3]*Department of Applied Medical Science, University of Southern Maine, Portland, ME and* [4]*Department of Chemical and Biological Engineering, University of Maine, Orono, ME.*

Nanoparticles, such as those made of silver, have shown great promise in a broad range of applications in industry and biomedicine due to their unique physical and chemical properties. Human exposure occurs in manufacturing settings as well as through consumer products and nanoparticles are being released into our environment. But whether these materials are safe for human exposure is not yet clear. It is essential to understand if and how nanoparticles induce damage in normal human cells. To address this issue, we investigated the cytotoxicity and genotoxicity of silver nanoparticles in human lung cells. We found that bare silver nanoparticles are toxic to human lung cells after 120 h exposure. There is no induction of chromosome damage or DNA double strand breaks in cells exposed to silver nanoparticles for 24 h, however, after 120 h exposure, we noted a weak induction of chromosome damage and DNA double strand breaks. Further work will include assessing cytotoxicity and genotoxicity of pegylated and fluoroscein isothiocyanate functionalized (FITC) silver nanoparticles to see if alterations to the base nanoparticles render them more or less toxic and determine if cellular transformation occurs after silver nanoparticle exposure.

PS 2165 INFLUENCE OF SURFACE FUNCTIONALIZATION ON PROTEIN ADSORPTION AND NANOPARTICLE UPTAKE BY INTESTINAL EPITHELIAL CELLS.

P. N. Wiecinski[1], K. M. Louis[2], R. M. Albrecht[3] and J. A. Pedersen[1, 4]. [1]*Molecular and Environmental Toxicology, University of Wisconsin-Madison, Madison, WI,* [2]*Chemistry, University of Wisconsin-Madison, Madison, WI,* [3]*Animal Science, University of Wisconsin-Madison, Madison, WI and* [4]*Soil Science, University of Wisconsin-Madison, Madison, WI.* Sponsor: R. Peterson.

The surface chemistry of nanoparticles (NPs) is expected to influence their uptake and bioavailability. Many engineered NPs are functionalized to improve dispersibility in aqueous media, enhance biocompatibility or facilitate incorporation into nano-composite materials. Additional coatings may be acquired during exposure to environmental and biological systems. Ingestion of NPs is expected to be an important exposure route to a variety of organisms, including humans. We have synthesized fluorescent europium-doped yttrium vanadate (YVO_4:Eu) NPs to examine the influence of surface chemistry on dietary protein adsorption and uptake using intestinal cell culture models. The benefits of YVO_4:Eu NPs include chemical stability under experimental conditions, low toxicity, the fluorescence of Eu facilitating tracking, and ease of functionalization to produce water-stable NPs. Here, we present results for citrate-stabilized and poly(acrylic acid) (PAA)-coated YVO_4:Eu

NPs. Aggregation of citrate-stabilized YVO_4:Eu NPs occurred at low pH (pH 2) and under high ionic strength conditions (i.e., PBS, cell culture media). However, PAA-coated YVO_4:Eu NPs remain stable without aggregation under these conditions. Surface coating affected adsorption of soy proteins (used as a simple model for dietary proteins) to YVO_4:Eu NPs as evidenced by differences in protein banding patterns determined by SDS-PAGE. Citrate-stabilized YVO_4:Eu NPs were taken up by Caco-2 cells, an intestinal enterocyte model, and detected in the cytoplasm by fluorescent microscopy. Inductively coupled plasma-optical emissions spectroscopy indicated uptake of citrate-stabilized YVO_4:Eu NPs is time- and concentration-dependent. Both fluorescence microscopy and ICP-OES data suggest NPs are either removed from or sequestered within the cells following longer exposure periods (≥ 2 hrs).

PS 2166 INTERNALIZATION OF CARBON BLACK AND IRON OXIDE NANOPARTICLE MIXTURES LEADS TO OXIDANT PRODUCTION.

J. Berg and C. M. Sayes. *Texas A&M University, Bryan, TX.*

Exposure to nanoparticle mixtures is increasing as nanomaterials enter consumer environments. In this study, the cellular effects of mixed engineered carbon black (ECB) and iron oxide (Fe2O3) nanoparticles was investigated in order to understand the mechanism of toxicity and potential methods of mitigation. Lung epithelial cells (A549) were exposed to mixed Fe2O3 and ECB (± L-ascorbic acid) and mixed Fe2O3 and surface-oxidized engineered carbon black (ox-ECB). All nanoparticles were characterized prior to experimentation using a variety of techniques. Cellular uptake of nanoparticles was analyzed via both fluorescence and transmission electron microscopy; cellular uptake of iron was quantified using inductively coupled plasma mass spectrometry. Both the MTT assay and the ethidium homodimer and calcein AM live/dead assay were used to measure cellular proliferation and cytotoxicity, respectively. The dichlorofluorescein diacetate assay was used to measure intracellular reactive oxygen species. Results show that both Fe2O3 and ECB (or ox-ECB) are co-internalized in intracellular vesicles. Additionally, after exposure to the mixture of nanoparticles, the lysosome (pH<5.2) quantity increased. Cellular uptake of Fe2O3 was unaffected by mixing with ECB. Significant oxidant production occurred in cells exposed to mixed Fe2O3 and ECB, but not in cells exposed to mixed Fe2O3 and ox-ECB, nor in cells exposed to Fe2O3 and ECB with ascorbic acid addition. Furthermore, exposure to mixed Fe2O3 and ECB exhibited a dose-dependent decrease in cellular proliferation and a decrease in cellular viability that were not seen in the Fe2O3 and ox-ECB mixture scenario. The results support the hypothesis that exposure to mixed Fe2O3 and ECB produces oxidants that are mediated by the surface reductive capability of ECB when both particle types are co-localized in acidic cellular compartments. This oxidant production mechanism can be mitigated by an anti-oxidant such as ascorbic acid, or by surface treatment of the ECB to decrease its surface reductive capacity.

PS 2167 *IN VITRO* PULMONARY TOXICITY OF METAL OXIDE NANOPARTICLES.

R. J. Snyder and K. Dreher. *U.S. EPA, Durham, NC.*

The diversity of engineered-nanomaterials and their applications as well as potential unknown health effects of these novel materials are significant challenges to assessing the health risks of nanotechnology. An integrated multi-tier testing strategy (www.epa.gov/nanoscience/) was implemented to address these challenges allowing for the rapid screening/prioritization of engineered-nanomaterials and ultimately identify alternative tests predictive of their toxicity. This research represents Tier 2 – in vitro toxicity assessment of 8 metal oxide nanoparticles (NPs). Human bronchial epithelial airway cell line (BEAS2B) was exposed for 24 or 48 h to various concentrations (6 – 100 μg/ml) of 6 TiO2 or 2 CeO2 NPs obtained from a variety of commercial sources and differing in size and crystal structure. The effect which NPs had on cell cytotoxicity was measured using both the WST-1 assay and LDH release. The ability of NPs to induce cellular reactive oxygen stress (ROS) was measured at 2h post-exposure by DCFH fluorescent intensity. TiO2 and CeO2 NPs had no significant dose-dependent effect on the BEAS2B cell cytotoxicity when examined using the WST-1 assay. Differential cell cytotoxicity amongst the various TiO2 and CeO2 NPs [CeO2(15nm) > TiO2(32nm/anatase-rutile) > CeO2(70nm) > TiO2(200nm/anatase) = TiO2(10nm/anatase) = TiO2(30nm/anatase-rutile) > TiO2(25nm/anatase) > TiO2(250nm/rutile)] was detected by LDH release as early as 24h post-exposure. BEAS2B cellular ROS was induced in differential and dose-dependent manner by all TiO2 NPs [TiO2(25nm/anatase) = TiO2(250nm/rutile) > TiO2(30nm/anatase-rutile) = TiO2(10nm/anatase) = TiO2(32nm/anatase-rutile) = TiO2(200nm/anatase) > CeO2(70nm) = CeO2(15nm)]. CeO2 NPs did not induce ROS in BEAS2B cells at any size or dose. The results demonstrate that differential toxicity of various TiO2 and CeO2 NPs was detected by in vitro pulmonary toxicity testing. Metal oxide NP BEAS2B cell cytotoxicity was found not to be dependent on size alone and did not correlate with the ability to induce ROS. This abstract does not necessarily reflect EPA policy.

PS 2168 GENE EXPRESSION OF METAL UPTAKE GENES, USING CDSE/ZNS QUANTUM DOTS IN ZEBRAFISH LIVER CELLS.

V. Allagadda, S. Tang and G. D. Mayer. *The Institute of Environmental & Human Health, Texas Tech University, Lubbock, TX.*

Use of nanoparticles, defined as particles that have at least one dimension of 100 nm or less, is increasing in commercial and medical products. Quantum dots are a type of fluorescent, semi-conductive nanoparticle that are used in applications from biological imaging to computer chip manufacture. Increasing use of these types of particles hastens the need to study their toxicological implications since the toxicological parameters of quantum dots are not well defined and the toxicological mechanisms of action are not known. Nanoparticle characteristics differ in size, surface charge, coating agents and surface chemistries which may influence toxicity of individual preparations of particles. This study focuses on multiple comparisons of the toxicities of CdSe quantum dots and cadmium or zinc salts in zebrafish at the molecular and cellular level. To determine whether particle composition or size plays an important role in nanocrystal toxicity, zebrafish liver (ZFL) cells were exposed to various concentrations of quantum dots and cadmium or zinc salts. Isolated mRNA from these exposures was used to measure the expression of metal related genes including MT, MTF, DMT, ZIP and ZNT. Gene expression from Cd or Zn exposures showed that all genes were significantly up regulated in a dose dependant manner. Three different sizes of CdSe/ZnS QDs were used to expose ZFL cells at concentrations of 25nM, 50nM and 100nM. CdSe/ZnS nanocrystals altered gene expression of metal homeostasis genes in a manner different from that of the corresponding Cd or Zn salts. This implies that toxicity of CdSe nanocrystals is not due to ionic metal toxicity from particle dissolution.

PS 2169 ACCUMULATION AND TRANSLOCATION OF NANOMATERIALS ACROSS THE HUMAN PLACENTA.

P. Wick[1], A. Malek[2], P. Diener[3], A. Zisch[2], H. F. Krug[1] and U. von Mandach[2]. [1]*Laboratory for Materials-Biology Interaction, Empa Swiss Federal Laboratories for Materials Science and Technology, St. Gallen, Switzerland,* [2]*Department of Obstetrics, University Hospital Zurich, Zurich, Switzerland and* [3]*Institute of Pathology, Kantonsspital St. Gallen, St. Gallen, Switzerland.* Sponsor: B. Fadeel.

Background and Objectives: Humans have been exposed to fine and ultrafine particles throughout their history. Since the Industrial Revolution, sources, doses, and types of na-noparticles have changed dramatically. In the last decade, the rapidly developing field of nanotechnology has led to an increase of engineered nanoparticles with novel physical and chemical properties. Regardless of whether this exposure is unintended or not, a careful assessment of possible adverse effects is needed. A large number of projects have been carried out to assess the consequences of combustion-derived or engineered nanoparticle exposure on human health. In recent years there has been a growing concern about the possible health influence of exposure to air pollutants during preg-nancy, hence an implicit concern about potential risk for nanoparticle exposure in utero. Previous work has not addressed the question of whether nanoparticles may cross the placenta.
Methods: We used the ex vivo human placental perfusion model to investigate whether nanoparticles can cross this barrier and whether this process is size dependent. Fluores-cently labeled polystyrene beads with diameters of 50, 80, 240, and 500 nm were chosen as model particles.
Results: We showed that fluorescent polystyrene particles with diameter up to 240 nm were taken up by the placenta and were able to cross the placental barrier without affect-ing the viability of the placental explant.
Conclusions: The findings suggest that nanomaterials have the potential for transplacental transfer and underscore the need for further nanotoxicologic studies on this important organ system.

PS 2170 CYTOTOXICITY AND BIOKINETICS OF POLYMERIC MAGHEMITE NANOPARTICLES *IN VITRO.*

V. Sorribas[1], L. Mohamed-Ahmed-Ali[2, 1], R. Villa-Bellosta[1], A. Millan[1], L. Gabilondo[2], R. Piñol[2] and F. Palacio[2]. [1]*Laboratory of Molecular Toxicology, University of Zaragoza, Zaragoza, Spain and* [2]*Insitute of Materials Science, CSIC, Zaragoza, Spain.* Sponsor: A. Anadon.

Biocompatible ferrofluids are stable suspensions of superparamagnetic nanoparticles (NP) that can be used in several medical applications, such as diagnosis, MRI contrast agents, targetted drug delivery, and as magnetothermic tools that respond

to a time-varying magnetic field to heat up specific targets like tumours. The ferrofluids used here consist of maghemite nanoparticles within a polivinylpyridine matrix covered with a functionalized polyethylenglicol coating, and dispersed in PBS at pH 7.4. Several samples have been prepared with hydrodynamic sizes from 50 to 100 nm and average maghemite nanoparticle sizes from 4 to 15 nm, comparable to those of biological entities of interest, and allowing to investigate size dependence of their relaxometric and magnetothermic response.
In order to assess the biocompatibility of NPs, cytotoxicity was studied in epithelial (Opossum kidney) and mesenchymal (vascular smooth muscle) cell lines. The activity of cellular lactate dehydrogenase in culture medium was determined as a function of dose, time and NP size. LC50 resulted to be dependent on all these parameters, including an accumulative effect on time. These findings were confirmed by ethidium bromide / acridine orange staining, in a fluorescence microscope. Cell death was not caused by an apoptotic response but by necrosis, most likely after lysosomal disfunction. Oxidative stress was also discarded as a toxic outcome. Uptake and subcellular fate of NP in the cells was analyzed using fluorophore-tagged NP, and co-localization assays with several subcellular structures including lysosomes, mitochondria, late and early endosomes, endoplasmic reticulum, cytoskeleton and plasma membrane. The endocytic route of cell uptake showed to be size-dependent and mediate through clathrin-coated endocytosis, as it was sensitive to K+-depletion and to chlorpromazine. Atomic absorption spectroscopy of cell iron content revealed that uptake was linear with time, and dependent on NP diameter and cell type.

PS 2171 TOXICITY ASSESSMENT OF NANOMATERIALS IN ALVEOLAR EPITHELIAL CELLS AT THE AIR-LIQUID INTERFACE.

Y. Xie, N. Williams, A. Tolic, J. Teeguarden, J. Pounds, A. Laskin and G. Orr.
Pacific Northwest National Laboratory, Richland, WA.

Airborne nanomaterials that enter the respiratory tract are likely to be deposited in the alveolar region, where alveolar epithelial cells are found at the interface with ambient air. To date, the majority of *in vitro* studies characterizing the interactions and impact of engineered nanomaterials in these cells have been carried out in cells submersed under growth media. To more closely mimic *in vivo* exposures, we have established the growth of alveolar type II epithelial cells (C10 cell line) at the air-liquid interface (ALI), enabling realistic exposures to aerosolized nanoparticles. This approach also supports accurate quantification of the delivered particles per cm^2 (or particles per cell) by collecting the particles on millimeter-size grids or glass cover-slips, placed randomly over the cells and visualized using electron or fluorescence microscopy, respectively. Cells, cultured on membrane inserts, have been grown at the ALI for at least 24 hours before being exposed to aerosolized nanoparticles, generated using a vibrating membrane nebulizer, for 10 minute sessions in an enclosed chamber that ensures uniform particle delivery. The cells have been kept at the ALI until assayed for lactose dehydrogenase (LDH) release to evaluate cell damage at 6 and 24 hours post exposure, and for proliferation rate (MTS) to evaluate viability at 24 hours post exposure. Using these conditions we found that exposures to 50 nm bare amorphous silica nanoparticles, containing embedded fluorescent dye molecules, elicit no significant cytotoxic response at concentrations ranging from 10 to 1000 particles per cell. These observations agree with observations we obtained in submersed cells exposed to equivalent doses, as estimated by a computational particokinetics (sedimentation, diffusion) model. Studies with surface modified aminated 50 nm amorphous silica nanoparticles and other particles that have shown to elicit toxic responses in submersed conditions are currently being pursued at the ALI.

PS 2172 *IN VITRO* CYTOTOXICITY AND OXIDATIVE STRESS INDUCTION OF GA-IN-CU-SE NANOPARTICLES IN HUMAN ALVEOLAR EPITHELIAL A549 CELLS.

J. Narvaez, M. Uribe-Ramirez, O. G. Aztatzi-Aguilar, O. C. Barbier and A. De Vizcaya-Ruiz. *Department of Toxicology, CINVESTAV IPN, Mexico City, Mexico.*

The exponential progress in engineered nanoparticles (NP) development urges the need to investigate their toxicity in view of the potential human exposure during handling, manufacture, and disposal. NP of $CuIn_{0.75}Ga_{0.25}Se_2$ (CIGS) are being used for the manufacture of solar (photovoltaic) cells with the purpose to improve power generation efficiency and reduce costs. Studies of cellular injury pathways related to the metallic nature of CIGS were performed: cytotoxicity (cristal violet assay), oxidative stress (production of reactive oxygen species (ROS) and catalase levels) and DNA damage (comet assay) after 24, 48 and 72h of exposure to CIGS (0 - 150μg/ml) in human alveolar epithelial A549 cells. Statistically significant loss of cell viability of 20 to 45% in a concentration-dependent was observed after 48 and 72h of exposure. No significant ROS generation was induced after CIGS exposure, although a slight increase with 10μg/ml was observed; in addition a significant increase in catalase levels was observed after 24h exposure to 10 and 150μg/ml-only. Damage to DNA was observed at 24h from exposure to all concentrations of CIGS. Damage by nanoparticles is linked to its size, composition and structure, potentially causing more damage with a larger surface area and capable to contain more chemical compounds. An interesting metal interaction of $CuIn_{0.75}Ga_{0.25}Se_2$ in the cellular environment is taking place, at lower exposure (10μg/ml) to CIGS Cu induced ROS and catalase, which seems to be neutralized at higher (50-150μg/ml) concentrations by Se, considering 2 parts of Se per part of Cu. Cellular toxicity response to CIGS showed their ability to induce cytotoxicity (cell death and DNA damage) and cytoprotective (catalase increase) responses, which in imbalance compromise cell homeostasis. Our study confirms the need to use toxicity testing strategies that target known cellular pathways and consider particular NP characteristics. (Acknowledgments: Dr V. Subramaniam and V. Bhojan for providing CIGS NP).

PS 2173 TOXICITY AND GENOMIC PROFILING OF ENGINEERED NANOMATERIALS WITH DISTINCT GEOMETRY AND SURFACE FUNCTIONALIZATION IN HUMAN AORTIC ENDOTHEIAL CELLS.

P. J. Moos, A. Mikkleson, H. Herd, G. Thiagrarjan and H. Ghandehari.
University of Utah, Salt Lake City, UT.

Manufactured nanoparticles have unique properties due to their size, shape, composition, surface area, and chemical composition. Nanoformulations of drugs can improve their efficacy and targeted delivery but less is known regarding potential adverse effects of these materials. Therefore, understanding potential toxicities related to these materials can lead to the engineering of safer nanomaterials. If new nanomaterials are to function in drug delivery, these agents are likely to have resident time in the vasculature and interact with endothelial cells. Since several nanomaterials have elicited pro-inflammatory responses, we sought to determine if nanosilica and dendrimers produced pro-inflammatory responses in primary human aortic endothelial cells (HAECs) as the promotion of inflammation may suggest that these materials may act as vesicants if used in medical applications. We have generated nanomaterials with distinct geometry and surface functionalization to evaluate the contributions of these parameters to the toxicology of these materials. For geometric evaluations, we generated 200 nm SiO2 nanospheres as well as nanoworms with a 200 nm diameter and lengths of ~1000 nm. While the overall toxicity was similar between the nanospheres and nanoworms, the nanoworms promote a distinct phenotype compared to the nanoSiO2 spheres in the HAECs. The nanoworms promote a vacuolization in the interior of the HAECs that is not apparent in the nanosheres. Surface functionalization was evaluated using cationic and anionic dendrimers composed of poly (amido amine) (PAMAM) with carboxylic acid or amine terminal groups, respectively. The amine terminated dendrimers are considerably more toxic than the carboxylated dendrimers as measured by cell viability assays. Using whole genome express profiling, we observe the induction of pro-inflammatory genes. Indeed many of the significantly upregulated genes are controlled by the inflammatory regulator NFκB.

PS 2174 SUBCELLULAR DISTRIBUTION OF GOLD NANOPARTICLES AND LACK OF CYTOTOXICITY IN RAT PRIMARY HEPATOCYTE CELL CULTURE MODEL.

Y. Zhang[1], W. Salminen[2], Q. Shi[2], X. Yang[2], Y. Jones[1], S. Linder[3], T. Mudalige[3], B. Miller[1], C. Weis[1] and P. Howard[1]. [1]*Office of Scientific Coordination, NCTR/FDA, Jefferson, AR,* [2]*Division of Systems Biology, NCTR/FDA, Jefferson, AR and* [3]*Arkansas Regional Laboratory, ORA/FDA, Jefferson, AR.*

Gold nanoparticles exhibit unique chemical and physical properties that are attractive for many biomedical applications such as drug delivery. More information is still needed to characterize its safety and efficacy of this nanoproduct. Gold nanoparticles were characterized using transmission electron microscopy (TEM), atomic force microscopy (AFM), Dark-field microscopy, Zetasizer, Nanosight, Nanophox, UV/Vis, Fluorescence, and Near-infrared spectroscopy (NIR). The potential cytotoxicity and subcellular distribution of Au NPs (10, 20, 50 nm, Nanocomposix) were investigated in rat primary hepatocyte cell. Cytotoxicity was evaluated by MTT, LDH, ATP assays. The generation of reactive oxygen species (ROS) was also assayed to assess oxidative stress responses. Morphological alteration and subcelluar localization of particles were examined by TEM, dark field high resolution illumination microscopy, and raman confocal microscopy. Data showed no cytotoxicity and no oxidative stress generated by gold nanoparticles; however, imaging analyses demonstrated cellular uptake of gold nanoparticles and localization within intracellular level. These results suggest that gold nanoparticles could be uptaken by rat hepatocyte cells but not cytotoxic under the exposure conditions. In addition, the multiple approaches used to characterize gold nanoparticles provided the important information to understand the biological responses of these particles.

PS 2175 THE ROLE OF SURFACE CHEMISTRY ON INTRACELLULAR OXIDANT GENERATION.

S. H. Ho[1], J. M. Berg[2], B. Guo[3] and C. M. Sayes[1, 2]. [1]*Department of Biomedical Engineering, Texas A&M University, College Station, TX,* [2]*Toxicology Program, Texas A&M University, College Station, TX and* [3]*Department of Mechanical Engineering, Texas A&M University, College Station, TX.*

As the push for technologically advanced products continues, the incorporation of nanotechnology-based material derivatives introduces a variety of properties (i.e. electrical, insulating, etc.) into products to help meet consumer demands. In this study, we examined the toxicological effects of mixtures of iron oxide (Fe_2O_3) and amorphous carbon black nanoparticles on human pulmonary epithelial cells (A549) and sprague dawley rats. Characterization of the A549 cell line revealed low levels of SOD (Mn & Cu/Zn), while both catalase expression and glutathione content (2.3 μM/10k cells) are seemingly high. Fe_2O_3 nanoparticles are 41 ± 17 nm in size with a negative zeta potential (-44.2 ± 17 mV). Amorphous carbon nanoparticles are 47 ± 7 nm. Results indicate that intracellular reactions between mixtures of Fe_2O_3 and engineered carbon black (ECB) generate reactive oxygen species (ROS) greater than exposures of either particle alone. Following intracellular oxidant production, cellular viability was assessed as measured by tetrazolium salt reduction as well as intracellular esterase activity after 24 hours exposure to the nanoparticle mixtures. Associations between an increased redox capacity of the amorphous carbon black and the decrease in cellular viability were noted. Changes in both the reductive capacity (9.0 x 10-3 to 2.4 x 10-4 g(Fe^{3+})/g(carbon)) and change in oxidant production were lessened after surface oxidation. The proposed role of ECB is to reduce disassociated Fe^{3+} to Fe^{2+}, allowing intracellular redox reactions to generate oxidants. Further work involving fullerene-based nanostructures with differing reductive capacities, due to differing surface modifications, as well as quantifying additional cellular biomarkers of oxidative stress will be examined.

PS 2176 NICKEL NANOPARTICLES AND PLATELET-DERIVED GROWTH FACTOR (PDGF) ACT COORDINATELY TO INCREASE PRO- AND ANTI-ANGIOGENIC FACTORS IN RAT PLEURAL MESOTHELIAL CELLS.

E. E. Glista, B. C. Sayers, A. J. Taylor and J. C. Bonner. *Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.*

Nickel nanoparticles (NiNP) are used as a catalyst in the manufacture of multi-walled carbon nanotubes (MWCNT). There is concern that NiNP or MWCNT could cause cancer or fibrosis in humans. We recently reported that inhaled MWCNT migrate to the pleura in mice to cause subpleural fibrogenesis, a disease process that involves angiogenesis. Fibrosis caused by MWCNT was accompanied by increased levels of platelet-derived growth factor (PDGF), a mediator of fibrosis. We hypothesized that NiNP and PDGF act coordinately to stimulate pleural mesothelial cells to increase angiogenic and profibrotic factors. In order to test this hypothesis, rat pleural mesothelial cells (NRM-2) were grown to confluence, rendered quiescent in serum-free medium, and then dosed with NiNP (10 μg/cm2) in the absence or presence of PDGF-BB (50 ng/ml). RNA was collected for Taqman quantitative real time RT-PCR to measure VEGF (a pro-angiogenic growth factor) and CXCL10 (an anti-angiogenic chemokine). RT-PCR results showed that NiNP alone caused a several-fold increase in CXCL10 or VEGF mRNA levels at 48 hr, while PDGF alone only marginally increased CXCL10 or VEGF mRNA levels. However, PDGF synergistically increased NiNP-induced CXCL10 and VEGF mRNA levels. The synergistic action of PDGF-BB on NiNP-induced VEGF was blocked by pretreatment with PD98059, a MAP kinase (ERK-1/2) inhibitor. Western blot analysis showed that PDGF also amplified NiNP-induced increase in the hypoxia-inducible factor (HIF)-1alpha, a transcription factor involved in VEGF production. PD98059 blocked PDGF-induced HIF-1alpha but not NiNP-induced HIF-1alpha protein levels. These data suggests that ERK-1/2 is important for PDGF-BB amplification of NiNP-induced HIF-1alpha signaling and VEGF production that leads to angiogenesis and fibrosis. However, PDGF also amplifies NiNP-induced CXCL10 that serves to reduce angiogenesis and fibrosis.
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PS 2177 CONTRIBUTION OF PHYSICOCHEMICAL PROPERTIES OF 4TH PERIOD METAL OXIDE NANOPARTICLES TO THEIR CYTOTOXICITY IN CULTURED HUMAN LUNG CELLS.

Y. Huang[1], C. C. Chusuei[2], S. Mallavarapu[2], C. Wu[1] and R. S. Aronstam[1]. [1]*Biological Sciences, Missouri University of Science and Technology, Rolla, MO and* [2]*Department of Chemistry, Middle Tennessee State University, Murfreesboro, TN.*

The cytotoxicity of well-characterized, nano-sized oxides of fourth period metals (Ti, Cr, Mn, Fe, Co, Ni, Cu, and Zn), each of which is widely used in industry, was assessed in a nontransformed human lung cell line (BEAS-2B). These nanoparticles are either spherical or nearly spherical, and approximately 10-60 nm in size. Toxicity expressed on the basis of particle mass dosimetry increased with atomic number, with the exceptions of Fe2O3 (lower toxicity than expected) and CoO (higher toxicity than expected). A similar trend was observed with particle specific surface area. Exposure to metal oxides alters the cellular redox state, leading to cellular injury as revealed by lipid peroxidation, DNA strand breaks, and membrane depolarization. Oxidative stress produced by exposure to metal oxide nanoparticles also perturbs certain cellular signaling pathways, induces the expression of cell death-related genes, and compromises the ability of cells to maintain a low concentration of intracellular calcium. Synergistic interactions between intracellular [Ca2+] and OS are a likely contributing factor. While [Ca2+] and OS affect the activity of each other, they can induce cell death by distinct pathways. To understand factors contributing to the initiation of oxidative stress, we measured the point of zero charge (PZC) of the particles. The PZC was highly correlated with the cytotoxicity potency of the metal oxide particles. We further measured ion dissolution of metal oxides at pH 4.7 and 7.4. Again, the degree of dissolution was related to toxic potential, raising the possibility that metal ion effects contribute to the observed cytotoxicity. These results provide a fuller picture of the multiple factors underlying the cytotoxic effects of metal oxide nanoparticles.

PS 2178 SURFACE MODIFICATION OF MULTI-WALLED CARBON NANOTUBES ENHANCES PDGF-INDUCED CHEMOKINE & GROWTH FACTOR PRODUCTION BY RAT PLEURAL MESOTHELIAL CELLS.

A. J. Taylor[1], C. K. Devine[2], E. E. Glista[1], B. C. Sayers[1], G. N. Parsons[2] and J. C. Bonner[1]. [1]*Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC and* [2]*Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.*

Carbon nanotubes are gaining increasing attention due to possible health risks from occupational and environmental exposures. We recently reported that inhalation of multi-walled carbon nanotubes (MWCNT) caused inflammation and fibrosis of the pleura in mice. MWCNT can be further modified by thin-film coating with metal oxides through atomic layer deposition (ALD). We hypothesized that ALD coating of MWCNT with aluminum oxide (Al2O3) would alter toxicity and gene expression in cultured rat pleural mesothelial (NRM2) cells. To test this hypothesis, confluent quiescent cultures of NRM-2 cells were treated with 10 mg/cm2 uncoated MWCNT (U-MWCNT) or Al2O3-coated MWCNT (A-MWCNT) in the absence or presence of PDGF (50 ng/ml), a growth factor that is elevated in the lungs of rodents exposed to MWCNT. We then collected mRNA for Taqman quantitative real time RT-PCR at various time points to measure CCL2 (monocyte chemoattractant), CXCL10 (anti-angiogenic factor), VEGF (angiogenic factor), and TGF-β1 (pro-fibrogenic factor). RT-PCR results showed that U-MWCNT increased CXCL10 mRNA levels ~10 fold at 24 hr, but did not increase CCL2, VEGF, or TGF-β1. A-MWCNT increased CCL2 marginally, CXCL10 8-fold, VEGF 4-fold, and TGF-β1 2-fold. PDGF alone increased CCL2 and CXCL10 ~2-fold, and the combination of PDGF and U-MWCNT increased CCL2, CXCL10, and TGF-β1 mRNAs above that observed for either agent alone. The greatest response in gene expression was seen with a combination of A-MWCNT and PDGF, which additively increased CCL2, VEGF and TGF-β1 mRNAs as compared to the combination of U-MWCNT and PDGF. These suggest that ALD surface modification of MWCNT with Al2O3 could increase the toxicity and pathogenicity of MWCNT.
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PS 2179 A COMPARISON BETWEEN ZINC OXIDE NANOPARTICLES AND ORGANIC UV FILTERS IN UV-IRRADIATED HUMAN SKIN AND IMMUNE CELLS.

P. F. Wright[1, 3], G. J. D'Costa[1, 3], S. J. O'Keefe[1, 3], B. N. Feltis[1, 2, 3], V. Muthusamy[1, 3], T. J. Piva[1, 3] and T. W. Turney[2, 3]. [1]*School of Medical Science, RMIT University, Bundoora, VIC, Australia,* [2]*Center for Green Chemistry, Monash University, Clayton, VIC, Australia and* [3]*Nanosafe Australia (www.rmit.edu.au/nanosafe), Melbourne, VIC, Australia.* Sponsor: J. Ahokas.

Sunscreens and cosmetics use photoprotection products that contain active compounds to reduce ultraviolet (UV) light exposure, including organic chemical filters and/or metal oxide physical filters. Metal oxide nanoparticles (NP) have recently been incorporated into sunscreens to increase aesthetic appeal, but little is known about their effects on skin or immune systems. We compared the cytotoxicity of 5 organic chemical filters, 3 zinc oxide (ZnO) physical filters (particle size 30, 80 & 200 nm) and zinc ions (Zn^{2+} as $ZnCl_2$) in human skin and immune cells. Immortalised keratinocyte (HaCaT) cells were exposed *in vitro* to filters (1-100 μg/mL) with/without UV radiation at 0.3x Minimal Erythemal Dose (MED). Human THP-1 monocytes and macrophages were exposed to filters with/without 1

MED of UVA. Cell viability was determined 24 hr post-irradiation by the MTS assay. Preliminary results indicate that there was no difference in cytotoxicity between nanosized or bulk ZnO (200 nm) and Zn^{2+} alone, in both sham- and UV-irradiated skin cells. However, Zn^{2+} and all ZnO filters were more cytotoxic in HaCaT cells than most organics, at doses >10 μg/mL. In immune cells, organics decreased viability at similar doses in both cell types. Comparatively, organics were equally cytotoxic to ZnO NP in monocytes, but less cytotoxic in macrophages. Following UVA exposure of macrophages, there was a trend towards increased cytotoxicity with Zn^{2+} and 30 nm ZnO NP. Conversely, the cytotoxicity of organic UVA blocker butylmethoxydibenzylmethane, but not organic UVB blockers, was partially reduced by UVA. Our data indicate that the relative cytotoxicity of ZnO NP in this *in vitro* test system is comparable to that of Zn^{2+} alone, and is similar to organic UV blockers.

PS 2180 TITANIUM NANOPARTICLES INDUCE EXPRESSION OF ADHESION MOLECULES IN VASCULAR ENDOTHELIAL CELLS.

S. Han, M. Toborek and B. Hennig. *Superfund Research Program, University of Kentucky, Lexington, KY.*

Atherosclerosis is a chronic inflammatory disease and still the number one cause of death in the United States. Numerous risk factors for endothelial cell dysfunction and the development of atherosclerosis have been identified, including air pollution and inhalation of ambient nano-size ultrafine particles. Recently, engineered nanoparticles (NPs) such as metal NPs have attracted a great deal of attention due to their potential applications. However, there are also great concerns for their potential to cause adverse health effects in vascular systems because engineered NPs are similar in size and characteristics in comparison to ambient ultrafine particles. Among a variety of metal NPs, titanium NPs are receiving increasing attention due to their wide range of applications and mass production. To test the hypothesis that metal NPs can induce adhesion molecules in the endothelium, endothelial cells were treated with metal NPs. Among selected metal NPs, titanium NPs strongly, and in a concentration-dependent manner, induced mRNA and protein levels of vascular cell adhesion molecule-1 (VCAM-1). In addition, monocyte chemoattractant protein-1 (MCP-1) mRNA levels were increased after exposure to titanium NPs in a concentration-dependent manner. Titanium NPs with two different surface area (45 vs. 210 m2/g) showed similar effects in the induction of VCAM-1 and MCP-1. Exposure of endothelial cells with titanium NPs and pharmacological inhibitors, e.g., a JNK inhibitor, SP600125 and a PI3K inhibitor, LY294002, resulted in a decrease of VCAM-1 and MCP-1 expression, suggesting both JNK and PI3K are critical mediators of these proinflammatory responses. Furthermore, disruption of lipid rafts by cholesterol depleting agents did not influence titanium NP-induced VCAM-1 expression. These data show that titanium NPs can induce a cardiovascular disease risk via increased proinflammatory responses, such as adhesion molecule expression in vascular endothelial cells. This work was supported in part by NIH/NIEHS grant P42ES007380 and the UKAES.

PS 2181 SILVER NANOWIRES INDUCED INFLAMMATION IN AN *IN VITRO* HUMAN ALVEOLAR LUNG MODEL.

N. M. Schaeublin[1], C. A. Estep[1], J. R. Roberts[2] and S. M. Hussain[1]. *[1]711 HPW; RHPB, Air Force Research Labs, Wright Patterson, OH and [2]CDC/NIOSH, Morgantown, WV.*

The goal of this study was to evaluate the biological response following inhalation exposure to silver nanowires (Ag-NWs). Nanomaterials have been shown to penetrate deep into the alveolar regions of the lung, so we used a human alveolar lung co-culture model previously established in our lab as our model for inhalation exposure. The Ag-NWs were synthesized by nanoComposix and were 4 and 20 μm in length, with a similar diameter of ~90 nm. Changes in cellular morphology and the cellular interaction of the Ag-NWs with the cells was assessed using ultra-resolution microscopy after a 24 h exposure to 200 ng/ml of the Ag-NWs. The cell morphology and interaction of the Ag-NWs demonstrated that both lengths of Ag-NWs were interacting with the cells, but normal morphology indicated that they were not toxic. In addition, the co-cultures were exposed to Ag-NWs (concentrations ranging from 0-200 ng/ml) and cell viability was assessed using MTS to evaluate mitochondrial function. Our viability assays indicated that both Ag-NWs did not alter cell viability and that there was no toxicity. In addition, inflammatory responses were evaluated using ELISA assays to determine the secretion of 12 different cytokines. Of the cytokines evaluated, the Ag-NWs demonstrated an increase in secretion of several of them including IL-6, IL-8 and Interferon Gamma indicating that the Ag-NWs caused inflammation. Therefore, while the Ag-NWs were not toxic to the cells, they did cause irritation and an inflammatory response.

PS 2182 EVALUATING THE TOXICITY OF NANOMATERIALS USING A 3D NEURONAL CO-CULTURE MODEL.

L. K. Braydich-Stolle and S. Hussain. *AFRL/RHPB, Wright-Patterson AFB, OH.*

Use of unicellular in vitro models to represent complex tissues, such as the brain, has received criticism since these models cannot provide a modest to often needed exact depiction of how a multi-cellular tissue responds. However, in vitro cultures are key tools for preliminary foundation studies that assess dosing ranges, probable mechanisms of toxicity and that lower animal use by refining techniques prior to progressing to in vivo studies. As a bridging model system, the use of cell matrixes and multi-cell co-cultures such as those including more tissue specific cells or their resident immune cells may provide a linkage between in vitro to in vivo responses. Major players within the body's immune system include localized phagocytic cells that patrol and remove foreign materials they encounter to include toxic nanomaterials. In some cases, specific proteins called cytokines will signal phagocytic cells to a particular site within the body to aid in an immune response. This study focused on establishing in vitro neuronal co-cultures, neurons and their microglia, to evaluate the response effects of nanoparticle (NP) exposures. Co-cultures of neurons and microglia were grown on poly-l-lysine coated plates in Matrigel to simulate a 3D environment. Following exposure to 40 nm and 1 μm manganese NPs, there was a greater decrease in cell viability in the unicellular cultures, compared to when microglia cells were present. In addition to cellular viability, gene expression changes were evaluated as well as secretion of dopamine, norepinephrine and glutamate. When these endpoints were compared between the unicellular and co-cultures there was a drastic difference in the response. This model demonstrated the importance of normal phagocytic cells within the matrix for evaluating the biological responses to NPs, and suggests that the development of in vitro model systems, which more accurately simulate in vivo conditions, should be considered in order to obtain an accurate evaluation of tissue sensitivity from NP exposures.

PS 2183 CYTOTOXICOLOGICAL AND PATHWAY-FOCUSED PERTURBATIONS IN DERMAL CELLS EXPOSED TO QUANTUM DOT SYSTEMS.

A. A. Romoser and C. M. Sayes. *VTPP-Toxicology, Texas A&M, College Station, TX.*

Quantum Dot (QD) nanocrystals have received attention due to their unique electronic and optoelectronic properties. QDs are increasingly being used for a wide variety of industrial and consumer-based applications, including biomedical imaging agents, inks, and solar panels. QDs may also pose risks to human health due to heavy metal exposure and/or the production of reactive oxygen intermediates. However, it is largely unknown which specific pathways or subcellular mechanisms of action are triggered as a result of exposure to QDs. First, inflammatory and non-inflammatory immune responses were measured in HDF cells by probing for protein and mRNA expression with western blots and pathway-focused gene profiling. Cellular effects from QD (CdSe/ZnS-COOH) exposure induced upregulation of apoptotic, inflammatory and immunoregulatory proteins including TNFα, IL-1B and IL-10. QDs also caused modulation of genes known to be associated with inflammatory (IL1-β), immune (IL-1, IL-6, IL-10), cellular stress (HMOX1), and apoptotic (CASP1, ADORA2A, NLRC4) responses. QDs caused dose (1 to 120 nM exposure concentrations) and time (8 hr vs. 48 hr) dependent cell death, as evidenced by resazurin-based metabolic activity assays. Differential cytotoxicity in HEK and HDF cells was seen over a range of exposure concentrations. By 48hrs, viability of HEK cells was reduced to 12% vs. 57% in HDFs when exposed to 120nM concentrations. Additionally, we probed the differential cytotoxicological and morphological response in HDFs exposed to cadmium-containing QDs (CdSe/ZnS-PEI) or microencapsulated QDs, as measured by fluorescence microscopy, metabolic activity, and western blots. Results show that microencapsulated QDs induced less toxic response as compared to unencapsulated QDs, while still offering the desirable optic characteristics. This data suggests that the cytotoxic response of cells exposed to these materials is dependent upon size and surface coating. In addition, the observed toxic response can be mitigated by altering these physical features.

PS 2184 *IN VITRO* DNA DAMAGE, MUTATION, AND CYTOTOXICITY AND SUBCHRONIC CELL GROWTH STUDIES OF QUANTUM DOT NANOPARTICLES.

J. Bartel[2], J. Treadway[2], J. Schatz[3] and S. Kim[1]. *[1]Design-for-Environment, Life Technologies, Foster City, CA, [2]Life Technologies, Eugene, OR and [3]Design-for-Environment, Life Technologies, Madison, WI.*

Quantum dots are engineered nano-materials whose composition and toxicology profile are wholly dependent on the method of synthesis. Because of their ease of use and unique advantages over traditional fluorescent probes, quantum dots have

seen rapid uptake in biological imaging applications. In this study, we have evaluated the potential of commercially available CdSe/ZnS-based quantum dots (Qdot® 655 ITK™ amino and Qdot® 655 ITK™ carboxyl) to induce cytotoxicity and DNA damage in CHO-K1, V79, Hep, THP-1, and Caco-2 cells using LDH release, trypan blue dye exclusion, and MTT vital stain methods and Comet assay at concentrations of 15 nM to 500 nM. These quantum dots were also evaluated in bacterial tryptophan reverse mutation assay (500 nM). Lastly, cell growth rate was studied during subchronic exposure of the quantum dots to MRC-5 and GM03440 cells (5-15 nM). At 3-, 24-, and 48-hour treatment points with LDH release method, the relative toxicity range was from 0% to 50% at 15 nM to 500 nM quantum dots. In the MMT cell viability experiment, the cell viability ranged from 100% to 25% at 15 nM to 500 nM quantum dots. In the trypan blue cell viability experiment, the viability range was from 100% to 60% at 15 nM to 500 nM of quantum dots. Quantum dots were not mutagenic with and without metabolic activation. No statistically significant changes in cell growth, population doubling or cell morphology were found for subchronic treatment of human primary skin or lung fibroblast cell lines with various Qdot® nanoparticles. In the Comet assay, no significant DNA damage was seen across all tested quantum dot types at 50 nM. Some DNA damage was observed at 500 nM, but was far below methyl methanesulfonate (MMS) control. Based on the study results described above, CdSe/ZnS-based Qdot® nanoparticles are non-toxic at concentrations used for most biological applications (10 nM).

PS 2185 GLOBAL GENE EXPRESSION PROFILING ANALYSIS OF FOOD DEPRIVED MATERNAL AND FETAL MICE LIVERS.

T. Ogawa[1, 2], R. Rakwal[1, 2], J. Shibato[2], C. Sawa[1, 2], T. Saito[1], A. Murayama[1], M. Kuwagata[2, 3] and S. Shioda[1, 2]. [1]*Anti-aging Medicine, Showa University, Tokyo, Japan,* [2]*Anatomy, Showa Unversity, Tokyo, Japan and* [3]*Laboratory of Pathology, Food and Drug Safety Center, Kanagawa, Japan.*

Accumulated human epidemiological evidence has led scientists to theorize that undernutrition during gestation is an important early origin of adult diseases. Animal models have successfully demonstrated that maternal diet could contribute to some of adult diseases. Nevertheless, the mechanisms by which maternal diet causes these long-term impacts remain poorly understood. Although undernutrition is harmful in pregnant women, calorie restriction is a strategy proven to extend healthy and maximum life span in adult. This opposite effect of nutritional condition might provide us a hint to understand the mechanisms. In this direction, we have initiated a study on global gene expression profiling (via DNA microarray) following undernutrition, between adult and fetal tissues. Pregnant mice (C57BL/6J) were exposed to food deprivation (FD) on gestation day (GD) 17, and caesarean section was performed on GD18. Control mice were supplied with chow ad libitum till sacrifice on GD18. Liver was collected from five control and six FD mothers. Fetal liver was collected from two fetuses from each mother. Total RNA extracted from mother and fetal livers for each control and treatment (FD) was pooled in each group (control and FD), prior to DNA microarray analysis (Agilent mouse whole genome 4 x 44K). Results revealed 3058 & 3126 up (>1.5 fold)- and down (<0.75 fold)-regulated genes, and 1475 & 1225 up- and down-regulated genes, in the maternal and fetal livers, respectively. Functional categorization of gene changes between mother and fetus revealed major changes in categories related to cellular and metabolic processes. Commonly up- and down-regulated genes were 500 and 376 in mother and fetus, respectively. Interestingly, 103 genes up-regulated in mother were down-regulated in fetus, whereas 108 genes down-regulated in mother were up-regulated in fetus. These results mark the beginning in our quest for understanding the true initiating mechanisms of impacts of maternal diet on adult health.

PS 2186 MATERNAL-CORD BLOOD LEVELS OF BISPHENOL-A (BPA) IN HUMAN AND FOLLOWING BPA ADMINISTRATION IN PREGNANT SHEEP.

V. Padmanabhan[1], S. Halubai[1], V. Almudena[1], C. Liao[2], S. E. Domino[1] and K. Kurunthachalam[2]. [1]*University of Michigan, Ann Arbor, MI and* [2]*Wadsworth Center, Albany, NY.*

Human exposure to BPA is a contentious public health issue. To translate detrimental effects of BPA in animals to humans, it is essential to achieve internal exposure levels of BPA in animal studies comparable to that found in human. We measured free and total BPA in maternal and cord blood samples obtained from pregnant women (n=20) at delivery and in maternal and umbilical arterial blood samples on day 65 and 90 of gestation in vehicle-treated (control) and BPA-treated (n=6/treatment/time point: 0.5 mg/kg BPA s.c. days 30-90 of gestation; term ~147 days) sheep. Earlier studies found that a 5 mg/kg BPA dose produces circulating levels of free BPA 2-3 times higher than the highest level seen at term in humans. Care was taken to minimize plastic contact. Free and total (after digestion with glucuronidase) BPA levels in plasma were quantified using Agilent 1100 series HPLC interfaced with an Applied Biosystems API 2000 electrospray MS/MS. Detection limit of BPA was 0.02 ng/ml (twice that of the valid lowest acceptable calibration standard). Findings revealed ~80% of BPA in human maternal circulation to be free (range 0.11 to 28.7 ng/ml; mean±SEM 4.2±1.6) and levels of BPA in cord blood to range between 1.1 to 62% of that seen in maternal blood. In contrast, majority of BPA in maternal and cord blood of sheep were in bound form with levels higher in day 90 than day 65 fetuses suggestive of fetal accumulation of BPA. Validation studies in sheep provided no evidence of BPA contamination. Differences in relative proportion of free and total BPA between day 65 and 90 may indicate differential processing of BPA during progression of pregnancy. Presence of large amounts of conjugated BPA in sheep and free in humans using identical detection methods suggests that free BPA in human is not due to the extraction procedure. Whether the predominance of free BPA in humans vs. conjugated in sheep is a function of timing (term in human and mid gestation in sheep) remains to be determined. (NIH ES016541 and ES17005 to VP).

PS 2187 ALZHEIMER'S DISEASE: EPIGENETIC INTERMEDIATES AND EARLY LIFE EXPOSURE TO PB IN PRIMATES.

S. W. Bihaqi[1], H. Huang[2] and N. H. Zawia[1]. [1]*Biomedical and Pharmaceutical Science, University of Rhode island, Kingston, RI and* [2]*School of Public Health, Zhengzhou University, Zhengzhou, Henan, China.*

A vast majority of AD cases (~90%) are sporadic, possess no clear genetic association and account for late onset AD (LOAD). The complex, non-Mendelian disease etiology of LOAD suggests that an epigenetic component could be possible. Our hypothesis states that early life exposure to toxicants such as lead (Pb) interferes with epigenetic pathways, thus resulting in late-life alterations in gene expression. To test this hypothesis, we studied the brains of Cynomolgus monkeys, which were orally dosed in 1980-81 with 1.5 mg/kg/day of lead-acetate (Pb) from birth to 400 days of age and vehicle thereafter. The monkeys were sacrificed 23 years later. Nuclear extracts were prepared from the frontal cortices of all groups, electrophoresed, transferred onto PVDF membrane, and probed with Dnmt1, Dnmt3a&b, MeCP2, Acetyl-Histone (Lys9/lys14) and Di-methyl histone H3K4 antibodies. Age dependent decreases in the protein expression of Dnmt1, 3a, 3b, MeCP2 were observed which were lowered in primates developmentally exposed to Pb. Protein expression of Acetyl-Histone (Lys9/lys14) and Di-methyl histone H3K4 showed an increase across the lifespan that was most significant at 23 years of age, whereas a significant decrease in the expression of the same was observed in primates developmental exposed to Pb. In conclusion, early-life exposure to Pb interferes with the levels of protein intermediates associated with epigenetic pathways in the aging brain. Future studies will correlate these changes with specific alterations in gene expression.

PS 2188 GESTATIONAL DIETHYLSTILBESTROL IMPACTS ADULT MALE PROGENY HEART STRUCTURE, FUNCTION, AND GENE EXPRESSION IN MICE.

R. Haddad, I. Sebag and L. Chalifour. *Lady Davis Institute, Montreal, QC, Canada.* Sponsor: K. Mann.

Rationale: Diethylstilbestrol (DES) disrupts endogenous hormone signaling. DES was prescribed to pregnant women exposing the fetus and was used in agriculture exposing the general public. Fetal cardiomyocytes express estrogen receptors suggesting that DES or other endocrine disruptors might impact the heart. We hypothesize that gestational DES alters cardiac structure /function and expression of proteins involved in calcium homeostasis. Methods and Results: Peanut oil (VEH) or DES (0.1, 1 or 10 mg/kg BW) was orally given to C57BL/6n mice (n=8) on gestational days 11-14. Adult male progeny were sedentary (SED) or swim exercised (SWIM) for 4 weeks. Echocardiography, electrocardiography and blood pressure measured cardiac function. Organ weights were collected at 4M. Immunoblots measured expression in heart homogenates. Baseline BW was reduced in DES10 and anogenital distance (AGD) increased after DES0.1 and DES1. Heart weight (HW) was increased in DES10. BW was reduced by SWIM in DES mice. HW was increased in VEH, unaffected in DES 0.1 and DES1, and reduced in DES10.0 by SWIM. Increases in SERCA2a (7-fold) and calsequestrin (4-fold) expression were

greatest in SED DES10. Phospholamban expression increased with DES. pSer16-PLB was reduced in all DES groups but pThr17-PLB had an inverted U response. After swim, SERCA2a increased in VEH and remained high with DES. CSQ and PLB were reduced; pSer16-PLB and pThr17-PLB levels increased. Systolic and diastolic blood pressure was significantly reduced after DES1. Fractional shortening and EKG did not change. SWIM increased the relative wall thickness only in VEHs. Conclusion: DES-mediated changes in calcium homeostasis due to altered cardiac gene expression led to an increased capacity for calcium transport and storage and the observed differences in cardiac mass and function in adult progeny. DES negatively affected the ability to accommodate swim. We conclude that gestational DES alters cardiac structure/function and expression of calcium homeostasis proteins in adult male progeny.

PS 2189 TESTOSTERONE-MEDIATED PROGRAMMING OF ADULT FEMALE MOUSE HEART.

W. Noiles, I. Sebag and L. Chalifour. *Lady Davis Institute, Montreal, QC, Canada.* Sponsor: K. Mann.

Rationale: Sex hormones are important for cardiac function. Fetal testes secrete testosterone and cardiomyocytes express androgen receptors leading to the idea that the fetal heart could respond to androgen programming. We hypothesize that gestational delivery of androgen will impact cardiac structure/function and calcium homeostasis expression in adult female progeny. Methods and Results: Pregnant C57Bl/6n mice (n=4) were injected subcutaneously with peanut oil (VEH) or testosterone proprionate (TP) during gestation days (GD) 11-14 (20ug, 200ug/kg body weight (BW)) or GD14-18 (2ug, 20ug, 200ug/kg BW). BW and anogenital distance (AGD) was measured. Echocardiography, EKG and blood pressure measured cardiac function. Organ weights were measured at 4M. Immunoblots of heart homogenates measured expression. BW was not significantly altered. Females treated GD14-18 with TP200 or GD11-14 with TP20 and TP200 had significantly longer AGD. At euthanasia female GD14-18 TP200 mice had significantly larger uteri due to a lack of vaginal opening. Mice after TP GD 14-18 injections failed to show significant changes in EKG parameters but TP treated GD 11-14 treated females had a significantly shorter QRS than VEH. Blood pressure was unaffected by treatment. GD 14-18 TP2 treated mice showed significantly reduced fractional shortening compared to VEH and a larger left ventricular inner-diameter compared with VEH, TP20 or TP200. All TP treated GD 14-18 females had significantly lower levels of the sodium-potassium ATPase and calreticulin than VEH. Significantly lower L-Type calcium channel was only detected in the TP2s. Conclusion: Gestational testosterone proprionate in the experimental design was adequate to masculinize female reproductive organs. Gestational TP significantly altered cardiac function and protein expression in GD11-14 and GD14-18 adult female progeny. These effects are mediated in a non-monotonic dose response, with the lower concentration of TP having a greater effect on cardiac function and the higher concentration a greater effect on expression. Gestational TP programs female fetal hearts.

PS 2190 EARLY LIFE PARTICLE AND OZONE INHALATION SENSITIZES THE LUNG AND BRAIN TO LATER LIFE CHALLENGES.

C. J. Johnston[1, 2], J. Allen[2], R. Gelein[2], J. N. Finkelstein[1, 2], G. Oberdörster[2] and D. A. Cory-Slechta[2]. *[1]Pediatrics, University of Rochester Medical Center, Rochester, NY and [2]Environmental Medicine, University of Rochester Medical Center, Rochester, NY.*

Epidemiological studies conducted in the United States and elsewhere suggest that childhood pulmonary diseases are increasing at an alarming rate. The EPA is required to set National Ambient Air Quality Standards (NAAQS) for particulate matter and ozone to protect public health with an adequate safety margin. However, these studies are done in the adult or aged animal models. We hypothesize that pulmonary exposure to ambient traffic related particles or ozone during postnatal lung development causes structural alterations and increased sensitivity to secondary challenges as adults. 4 day old C57Bl/6J mice were exposed to ambient "Real World" ultrafine and fine particles or ozone to examine how early life exposures effect re-exposure as adults. Groups of 4 day old mice were exposed for 4 hours a day for 4 consecutive days for 2 weeks and allowed to recover until 8 weeks of age. Mice were either sacrificed or re-exposed for 4 days and examined at the end of exposure. Endpoints examined were respiratory and CNS inflammatory markers. In addition, translocation of inhaled nanoparticles to the CNS and other secondary organs was determined. Microarray analysis demonstrated that re-exposure as adults induced mRNAs encoding cell cycle, antioxidant, proinflammatory cytokines and chemokines. Pulmonary lavage data demonstrated an increased neutrophilic influx in the lungs of challenged mice as compared to those only exposed as adults. Inhalation of gold showed greater accumulation in the kidney and spleen of re-exposed adults. Pollutant exposure during postnatal lung development demonstrated enhanced responses after re-challenge as compared to age matched mice which were only exposed as adults. Our results demonstrate increased sensitivity indicating that the lung is damaged or primed by earlier events, so exposure to a non-toxic dose of an environmental pollutant will trigger adverse responses.
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PS 2191 INTERSPECIES APPROACH TO THE ASSESSMENT OF HUMAN SUSCEPTIBILITY TO PHTHALATE-INDUCED ENDOCRINE DISRUPTION.

N. Heger[1], S. J. Hall[1], M. A. Sandrof[1], E. V. McDonnell[1], J. B. Hensley[4], K. J. Johnson[3], E. Houseman[2], K. W. Gaido[4] and K. Boekelheide[1]. *[1]Pathology and Lab. Medicine, Brown University, Providence, RI, [2]Community Health, Brown University, Providence, RI, [3]duPont Hospital for Children, Wilmington, DE and [4]The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

A testicular dysgenesis syndrome has been proposed, suggesting that alterations to the in utero and perinatal hormonal environment may explain temporal increases in male reproductive tract abnormalities. In utero exposure of male rats to the plasticizer di-(n-butyl) phthalate (DBP) reveals suppression of fetal testicular steroidogenesis, while mice remain relatively unaffected. To examine this species-specific sensitivity, a rodent host bioassay consisting of rodent and human fetal testicular grafts was developed. To validate the bioassay, fetal mouse (gd15) or rat (gd16) testes were xenografted into the renal subcapsular space of nude rat or mouse hosts, exposed to 250mg/kg/d DBP for 1, 2, or 3 days, and harvested 6 hours after the final dose. In DBP-exposed hosts, an increase in multinucleated germ cell (MNG) content was observed in both rat and mouse xenografts, with only rat xenografts exhibiting suppressed steroidogenic gene expression, consistent with the intact response. DBP treatment did not affect germ-cell content, and host species did not influence histopathology or gene expression endpoints. To explore the human response, a similar exposure paradigm was used, xenografting fetal testes (gestational weeks 10-23, n=17) into nude rat hosts. A significant increase in multinucleated germ cell content was observed following DBP exposure. RT-PCR analysis of steroidogenic genes revealed no DBP-induced alterations on any day. Taken together, these findings suggest that the human fetal testis responds more like the mouse than the rat following developmental phthalate exposure, and that the resulting effects on seminiferous cords and steroidogenic gene expression are mechanistically distinct, species-specific and intrinsic to the testis.

PS 2192 LACK OF TRANSGENERATIONAL EFFECT FOR VAGINAL PATENCY AND UTERINE WEIGHT IN CD-1 MICE EXPOSED TO DIETHYLSTILBESTROL OR 17BETA-ESTRADIOL.

E. Carney, R. J. Rasoulpour, M. J. LeBaron, J. Murray, R. Sura, J. Passage, R. Ellis-Hutchings and B. Gollapudi. *The Dow Chemical Company, Midland, MI.*

Epigenetic modifications, defined as heritable changes in gene expression via mechanisms other than changes in DNA sequence, can be passed through the germ line to influence transgenerational inheritance. Recent studies suggested that chemical exposure of pregnant mice 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 ~10 µg/kg/day diethylstilbestrol (DES) or ~30 µg/kg/day 17beta-estradiol (E2) via the diet or subcutaneous injection (SC) from gestation day 9 – lactation day (LD) 20. F1 offspring were mated for two additional generations, with F1-F3 offspring evaluated for uterotrophic effects on postnatal day (PND) 21 and vaginal patency. Exposed F0 dams in all groups had a 33-42% increase in LD 21 relative uterine weight. The F1 generation had a 3-4-fold increase in relative uterine weight for both DES and E2 diet groups; however, no effect was observed when DES and E2 were administered SC. All F1 groups had a treatment-related acceleration of vaginal patency. In addition, the F1 females from the DES SC group were subsequently found to be infertile, precluding further evaluation of this group. Surprisingly, there were no grossly observable uterine tumors at the 12-month interim necropsy conducted on a subset of the F1 generation; however histopathology and 18-month terminal tumor incidence data are pending. In the F2 generation, there was a marginal increase in mean PND 21 uterine weights for the dietary groups, but no indication of uterotrophic effects in the E2 SC group. Unlike the first generation, there was no effect on vaginal patency in the F2 litters. In the F3 generation, there was no

effect on uterine weight or vaginal patency in any treatment group. Results from this three-generation study thus far do not provide evidence for a transgenerational epigenetic inheritance phenomenon.

PS 2193 MATERNAL DHA SUPPLEMENTATION ATTENUATES HYPEROXIA-INDUCED LUNG DEVELOPMENTAL DEFICITS IN ADULT MICE.

L. K. Rogers[1, 2], K. Dingess[1, 2], M. Velten[1] and T. E. Tipple[1, 2]. [1]*Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH and* [2]*Department of Pediatrics, The Ohio State University, Columbus, OH.*

Preterm infants often require life-saving supportive therapies such as supplemental O2. Newborn mice exposed to hyperoxia have demonstrated decreased alveolarization similar to newborn infants. Docosahexanoic acid (DHA) is a long chain fatty acid that attenuates inflammatory responses. We tested the hypothesis that DHA administration to pregnant and nursing dams would decrease inflammatory responses and preserve lung growth which would result in improved lung function at 28 days of age. Dams were fed a purified control or DHA-supplemented diet beginning at E16. After birth, the pups were exposed to >85% O2 or RA for 14 days. After 14 days, both groups were returned to standard diets and RA until 28 days. Lung structure was assessed by morphometric analyses and pulmonary function was assessed by FlexiVent (SCIREQ). Protein levels of COX-2 and solulable receptor for advanced glycation end products (sRAGE) were analyzed by western blot. Mice that were DHA supplemented and exposed to hyperoxia during the newborn period demonstrated 1) increased alveolarization (alveolar number, 103.9±1.9 vs 65.1±3.5; alveolar area, 1516±51 vs 2058±69 microns2) and 2) improved lung function (tissue elastance, 24.9±1.6 vs 32.0±2.1 ml/cmH2O; tissue compliance, 0.042 vs 0.035 ml/cmH2O, DHA vs control diet, respectively) compared to the control diet hyperoxia exposed mice at 28 days of age. Neonatal hyperoxia exposure caused a sustained increase in COX-2 and sRAGE protein expression at 28 days that was attenuated in the DHA supplemented mice. Hyperoxia exposure during the neonatal period causes sustained lung deficits in adulthood. DHA administration to the dam dampened inflammation in the pups and improved lung growth and function in adulthood potentially though through RAGE or COX-2 mediated signaling pathways. We speculate that maternal DHA supplementation will be a beneficial intervention in preventing lung disease in premature infants.

PS 2194 PRENATAL AND POSTNATAL CIGARETTE SMOKE EXPOSURE INCREASES SUSCEPTIBILITY TO ADULT ONSET DISEASE.

J. L. Lyon and T. Gordon. *Environmental Medicine, New York University School of Medicine, Tuxedo, NY.*

Rationale: The CDC reports that an estimated 10-12% of the women who delivered a child, smoked during pregnancy, and of the 45% of female smokers that quit during pregnancy, more than half relapsed after delivery, potentially exposing more than 2 million babies to cigarette smoke (CS) during early development. CS is a complex chemical mixture with carcinogenic properties, and many of the chemical constituents can pass the placental barrier. CS condensate has been shown to alter the methylation patterns of DNA, potentially altering gene expression patterns. Given the delicate nature of fetal and neonatal development, CS exposure during these periods may change the expression patterns of critical genes, predisposing the offspring to diseases later in life. This study investigates the relationship between prenatal and early postnatal CS exposure and adult onset disease. Methods: Pregnant C57BL/6J mice were exposed via whole body exposure to 5mg/m3 mainstream CS for 3 hrs/day, 5 days per week during gestation. For postnatal exposures, the offspring were exposed to 5 mg/m3 environmental tobacco smoke for 3 hrs/day, 5 days/ week, from day 4 post partum until day 23 post partum. Total RNA was extracted from the liver, and hybridized to Affymetrix Mouse Gene 1.0 ST arrays to analyze differential gene expression. Results were confirmed using real time RT-PCR. Results: Prenatal and postnatal CS exposures resulted in gene expression changes. A predominance of upregulation was noted in both groups. The apoptosis related genes growth arrest and DNA damage inducible gamma 45 and polo-like kinase 3 were found significantly upregulated in both exposures. Two genes involved in the bioavailability of insulin like growth factor, insulin-like growth factor binding protein (IGFBP) 1 and 2, were upregulated in the postnatal (IGFBP-1) and prenatal groups (IGFBP-1 and IGFBP-2). Conclusion: Prenatal and postnatal CS exposure resulted in changes in gene expression in pathways that could result in adult onset diseases such as cancer and diabetes.

PS 2195 A DIRECT LC/MS/MS METHOD FOR DETERMINATION OF β-ALANINE IN HUMAN PLASMA.

C. Chen, C. Cunningham and J. E. Eble. *Critical Path Services, Garnet Valley, PA.* Sponsor: A. Chappelle.

Beta-alanine, a non-essential amino acid, is an important factor in building up muscle mass and strength. Abnormal beta-alanine levels in human diet have been reported to cause diseases such as cardiovascular disease, or symptoms such as paraesthesia, resulting from high blood-plasma concentrations of beta-alanine. Therefore, monitoring beta-alanine levels in human plasma samples may play a role in disease diagnosis and prevention. An LC-MS/MS method has been developed for use in this monitoring. The method can detect beta-alanine at level of detection (LOD) of 2 ppb in water. The limit of detection and quantification (LOQ) in plasma will be presented. Quantitative analysis of molecules below 100 daltons can be a challenge. Most of the existing methods for amino acid analysis involve a derivatization step, which is time-consuming. When electrospray ionization (ESI) is used as the ionization method, plasma samples present an additional challenge as they contain phospholipids which can result in matrix effects, specifically ion suppression. The rapid resolution method presented here uses an internal standard rather than derivatization for quantitative analysis. The instrument utilized is an Agilent 1200 HPLC and 6410 Triple Quadrupole MS equipped with a hot box upgrade operated in positive mode.

PS 2196 DIFFERENTIAL EFFECTS OF ADULT *VERSUS* PRENATAL HG EXPOSURE ON THE INNATE IMMUNE RESPONSE TO CVB3 INFECTION.

K. L. Speiran[1], D. L. Shirley[1], E. K. Silbergeld[2] and J. F. Nyland[1, 2]. [1]*Pathology, Microbiology & Immunology, University of South Carolina School of Medicine, Columbia, SC and* [2]*Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Exposures to mercury (Hg) compounds alter the immune system in adults; prenatal exposures alter the ontogeny of the immune system. We examined the effects of exposures to Hg in adults and in fetuses, and interactions of these exposures to later infection by Coxsackievirus (CVB3), a common infection that can, in susceptible individuals, lead to autoimmune myocarditis. Because of the role of matrix metalloproteinase (MMP) activation in the development of the dilated heart phenotype observed in the end stages of autoimmune myocarditis we examined effects of Hg on MMP as well as on IL-6 and IL-1β, cytokines that are involved in MMP activation. In the first study, exposure of adult female mice to Hg changed the innate immune response to CVB3 and increased subsequent severity of chronic autoimmune myocarditis. FACS analysis showed that this was accompanied by downregulation of costimulatory molecule (CD80/86) and upregulation of TLR4 expression on peritoneal macrophages and mast cells during the innate immune response. Further, MMP-9 expression was decreased in splenic macrophages. IL-6 levels in the spleen were elevated. In the second study, we examined the consequences of prenatal exposure to Hg and later responses to CVB3. Prenatal exposure to Hg changed the ontogeny of the immune response and subsequent response to adult infection. Prenatally exposed females downregulated TLR4 and upregulated MMP-9 expression in splenic macrophages following CVB3 infection. These results demonstrate the important role of the developmental state of the immune system on the immunotoxic effects of Hg. They suggest that Hg exposure in utero may change susceptibility factors relevant to adult immune response to infection. Funding provided by NIEHS.

PS 2197 NEONATAL GENISTEIN EXPOSURE CAUSES IMPLANTATION FAILURE BECAUSE OF PERMANENT ALTERATIONS IN UTERINE MORPHOLOGY AND STEROID HORMONE RESPONSES.

W. Jefferson, S. M. Wagner, E. Padilla-Banks and C. J. Williams. *NIEHS, Research Triangle Park, NC .*

Approximately 20% of US infants consume soy-based infant formulas and have high serum levels (1-5 uM) of the phytoestrogen genistein. Female mice exposed neonatally to genistein are infertile in part because the uterus does not support implantation. We hypothesize that genistein treatment permanently disrupts gene expression and leads to morphological abnormalities in the uterus that are inconsistent with successful implantation. Dramatic differences in uterine morphology were observed in genistein treated mice, including excessive deposition of extracellular matrix, minimal endometrial stroma, lack of glandular epithelium, and abnormal-appearing luminal epithelial cells. Microarray analysis was performed on uterine mRNA from control and genistein-treated mice on days 2 and 4 of pregnancy.

Of the >40,000 genes, 682 and 1,514 were significantly altered on day 2 and day 4, respectively. Many are known to affect uterine morphology and function, including transcription factors, secreted signaling molecules, hormonally responsive genes, and immune response genes. To validate and extend the microarray findings, real time RT-PCR was performed on altered genes at multiple time points from neonatal genistein treatment to early pregnancy. To test alterations in hormone responsiveness, control and genistein-treated mice were ovariectomized and then treated with estradiol or progesterone. These studies suggested that abnormalities in expression of transcription factors (FoxA2, Hox) and secreted signaling factors (Wnt7a) during neonatal treatment led to permanent alterations in uterine morphology. Furthermore, uterine hormone responses were dampened and/or delayed. Taken together, these data suggest that exposure to genistein during female reproductive tract development contributes to infertility by permanently altering gene expression that controls normal uterine morphology and critical cellular responses to hormonal cues of pregnancy. These findings may have implications for the use of soy-based infant formula and subsequent female reproductive health.

PS 2198 NEONATAL GENISTEIN EXPOSURE PERMANENTLY DISRUPTS OVIDUCT GENE EXPRESSION AND TISSUE ARCHITECTURE DURING PREGNANCY.

E. Padilla-Banks, W. Jefferson, S. M. Wagner and C. J. Williams. *NIEHS, Research Triangle Park, NC .*

Approximately 20% of US infants consume soy-based infant formula and have high circulating levels of genistein (GEN). Mice exposed neonatally to similar serum GEN levels are infertile due in part to abnormal embryo development in the oviduct. Histological analysis of the oviduct on day 2 of pregnancy revealed abnormal vascular development, muscle disorganization and excessive epithelial proliferation. Many epithelial cells were abnormally distended with intracellular mucopolysaccharide material. Microarray analysis identified 1126 oviductal genes significantly altered by neonatal GEN treatment, including transcription factors, signaling molecules, and vascular development factors. To validate and extend the microarray findings, real time RT-PCR was performed on oviducts collected on postnatal day 5 (during treatment) and several additional time points through day 4 of pregnancy. The transcription factors Pitx1, Nkx3.1, and Six1 were aberrantly expressed at all of these time points. Pitx1 and Six1 protein overexpression was confirmed by immunohistochemistry and immunoblotting. In contrast, Wnt7a was significantly downregulated during neonatal GEN treatment. The documented role of Wnt7a in female reproductive tract development suggests that Wnt7a downregulation in the neonatal period may explain some of the morphological abnormalities observed. To determine how the altered oviductal environment impacted preimplantation embryo development, embryos flushed from oviducts/uteri on days 1-4 of pregnancy were analyzed. Fertilization was significantly delayed in GEN treated mice, but developmental timing was recovered by the blastocyst stage. Significantly, there was a lower trophectoderm/inner cell mass ratio in blastocysts from GEN treated mice, suggesting that the abnormal oviductal environment affected blastocyst differentiation. This alteration did not affect the number of live pups delivered following blastocyst transfer experiments. Taken together, these findings have implications for the use of soy-based infant formula and subsequent female reproductive health.

PS 2199 DELAYED TEMPORAL INCREASE OF HEPATIC HSP70 IN APOE-/- MICE WITH ACCELERATED ATHEROSCLEROSIS INDUCED BY *IN UTERO* ARSENIC EXPOSURE.

N. N. Ngalame, M. E. Feil, A. F. Micciche and J. States. *Pharmacology/Toxicology, University of Louisville, Louisville, KY.*

Early life toxic exposures can predispose to adult disease. Chronic arsenic (As) exposure is associated with increased cardiovascular disease (CVD). We showed that in utero As exposure accelerates atherosclerosis underlying CVD in ApoE-/- mice, but the mechanism is unknown. As is a hepatotoxicant, and liver disease increases atherosclerosis risk. We hypothesize that in utero As exposure primes the liver for susceptibility to other environmental insults, predisposing to liver disease and accelerated atherosclerosis. Microarray analyses showed As increased Hsc70 and Hsp70 mRNA expression in livers of 10 wk old mice. We examined developmental expression of Hsc70 and Hsp70 and effects of As on DNA methylation in liver. Pregnant mice were given drinking water with 85 mg/L NaAsO2 (49 ppm As) from gestation day (GD) 8 - 18. Livers were obtained from GD18, and 3, 10 and 24 wk old mice. As content in GD18 liver analyzed by ICP-MS was 350 ng/g indicating As crossed placenta and reached fetal liver. Age-dependent Hsc70 and Hsp70 expression and global DNA methylation were determined by western blot analyses and methyl acceptance assay respectively. Gene-specific methylation was determined in 10 wk old livers by bisulfite sequencing. As did not alter expression of Hsc70. However, As induced Hsp70 at age 3 wk with increase at 10 wk, but return to unexposed levels by 24 wk. Global DNA methylation was age-dependent, with As having no effects. To determine if increased Hsp70 expression was due to epigenetic effects of As, we analyzed Hsp70 promoter region and part of its CpG island. The promoter region was 100% unmethylated in DNA of exposed and unexposed mice. However, CpG island showed differential methylation with region-specific hyper- and hypomethylation relative to unexposed mice. These results show in utero As exposure induces temporal Hsp70 expression increase, suggesting a temporal state of stress in the livers which can predispose the mice to developing atherosclerosis. Supported in part by USPHS grants ES015812 and ES014443.

PS 2200 CORRELATION BETWEEN POLYBROMINATED DIPHENYL ETHERS (PBDES) AND PERSISTENT ORGANIC POLLUTANTS (POPS) OR HEAVY METALS DETECTED IN UMBILICAL CORDS.

Y. Igoshi[1], S. Ochiai[1], E. Todaka[1, 2], Y. Matsuno[1], S. Suzuki[4], N. Shimojo[3], Y. Kohno[3] and C. Mori[1, 2]. *[1]Bioenvironmental Medicine, Chiba University, Chiba City, Chiba, Japan, [2]Preventive Medical Science, Chiba University, Chiba, Japan, [3]Pediatrics, Chiba University, Chiba, Japan and [4]Pediatrics, Shimoshizu National Hospital, Yotsukaido, Japan.*

The purpose of this study was to clarify the relationship between the levels of polybrominated diphenyl ethers (PBDEs) and persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), pesticides, and heavy metals in human umbilical cord. Eighty-six umbilical cords were collected at a maternity clinic in Chiba City, Japan, and PBDEs, POPs and heavy metals in the umbilical cords were analyzed by GC/MS. As the results, PBDEs, PCBs, lead, mercury, p,p'-DDT, p,p'-DDE, trans-nonachlor, oxychlordane, HCB, mirex, and β-HCH were detected from all the samples. Significant correlation (P<0.01) among POPs were found. The coefficient of determination (r2) between PCBs and p,p'-DDT, p,p'-DDE, trans-nonachlor, oxychlordane, HCB, mirex, and β-HCH were 0.508, 0.612, 0.340, 0.446, 0.504, 0.559 and 0.441, respectively. However, correlation between PBDEs and POPs or between PBDEs and heavy metals was not found. This was estimated because of the difference of exposure sources and half life time of chemicals and heavy metals in human body. Further studies are required to find the relationship between children's health and fetal exposure to POPs, heavy metals and BFRs.

PS 2201 *IN UTERO* AND POSTNATAL EXPOSURE TO FLUORIDE PRODUCES HYPERINSULINEMIA AND IMPAIRED GLUCOSE TOLERANCE IN RAT.

L. M. Del Razo, J. G. García-Lopez, A. Barrera-Hernandez and B. Olivier. *Toxicology, Cinvestav-IPN, Mexico, Distrito Federal, Mexico.*

Endemic fluorosis is now known to be global in scope, occurring on all continents and affecting many millions of people through the intake of groundwater fluoride contaminated by geological sources. The most significant risks are the development of skeletal fluorosis, and toxic effects on neurological, renal, endothelial, and gonadal tissues. Decreased insulin gene expression and protein secretion, as well as intolerance glucose, have been associated with high level of fluoride exposure. As xenobiotics exposure during prenatal stage can contribute to the adult disease. In this study, pregnant rats were exposed to fluoride through drinking water (50 mg/l) from day 2 to term, and effects were assessed in offspring at adult age for 114 days of postnatal fluoride exposure(50 mg/l). Fasting blood glucose (FBG) and increase in 2-hour blood glucose (2HBG) recorded during oral glucose tolerance test were monitored during the study. We have, therefore examined serum insulin concentration. Our results show no significant differences in FBG during the study. However a marginal increase in 2HBG was observed in offspring exposed (121.13 ± 6.05 vs 107.89 ± 5.91 mg/dl; p= 0.003). Notably, fasting plasma insulin levels were significantly higher (4.0 ± 0.95 vs 1.14 ± 1.02 μg/l; p= 0.004) in offspring exposed. These results suggest a pre-diabetic effect due to in utero and postnatal exposure to fluoride.

PS 2202 METABOLOMICS: INVESTIGATING THE IMPACT OF GESTATIONAL EXPOSURE TO A PHTHALATE ON THE BRAIN AND REPRODUCTIVE ORGANS OF THE DAM AND PREPUBERTAL PUPS.

R. Banerjee[1], R. Snyder[2], W. Pathmasiri[2] and S. J. Sumner[2]. *[1], University of North Carolina Chapel Hill, Chapel Hill, NC and [2]RTI International, Research Triangle Park, NC.*

The potential for human exposure to phthalates derives from their use in the production of industrial and household products. The Center for Disease Control reports measurable levels of phthalate metabolites in the general U.S. population, in-

dicating widespread exposure. While health effects from phthalate exposure are not understood, phthalates have endocrine disruption potential and have been implicated as contributing to the obesity epidemic. Our study was conducted to reveal the impact of in utero exposure to a phthalate on the biochemical profiles of brain and reproductive organs from prepubertal pups born to dams administered butylbenzyl phthalate (BBP). Pregnant rats were exposed by gavage daily during gestation (gd 14-21) to vehicle or to BBP in vehicle at a level known to induce effects in the offspring and at a level previously not shown to induce effects. Traditional phenotypic anchors (e.g., anogenital distance, retained nipples) were measured in pups (between pnd 0 and pnd 26). Tissues were collected from the dam on post natal day (pnd) 21 (~3 wks after BBP administration), and from pnd 26 pups (~ 4 wks after birth to dosed dams) and processed for metabolomics analysis. Student's t-tests were used to compare levels of metabolites among the BBP- and vehicle- exposed groups, and multivariate analysis was used to determine metabolites that best separate groups. Using t-tests, significant differences (control vs BBP groups) in levels of metabolites in brain tissue (male and female) and testes were found for pups, but not for dams. Principle component analysis and partial least squares projection to latent structures discriminant analysis were applied to the metabolomics data and revealed metabolites most important to the separation of all study groups. Overall-the major metabolic perturbations were related to metabolic pathways which include amino acids. This study demonstrates the use of metabolomics to reveal metabolic perturbations in tissues of offspring following in utero exposure to chemicals.

PS 2203 *IN UTERO* BAP-EXPOSURE LEADS TO DYSREGULATION OF THE CARDIOVASCULAR SYSTEM OF LEH RAT OFFSPRING IN LATE LIFE.

G. E. Jules[1], M. M. Maguire[1], A. Ramesh[2] and D. B. Hood[1]. [1]*Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN and* [2]*Biochemistry and Cancer Biology, Meharry Medical College, Nashville, TN.*

Cardiovascular disease (CVD) affects over 71 million American adults and remains the leading cause of death throughout Europe and the United States. Adult lifestyle choices, nutritional habits, and levels of physical activity significantly contribute to the development of CVD, which may be associated with intrauterine growth or early events in childhood. An increasing body of evidence indicates that transplacental exposure to PAHs at relatively high concentrations is linked to adverse birth outcomes. Benzo[a]pyrene (BaP), a prototypical PAH, is known to cause a number of effects, including atherogenic and cytotoxic effects in various species and tissues. However the mechanisms of action by which prenatal BaP exposure results in adverse effects on the development of fetuses and young children is not fully understood. In this study, we proposed that prenatal exposure of Long Evans Hooded (LEH) rat dams to BaP will result in deficits in cardiovascular development. Time-pregnant LEH rat dams were treated with 600ug/kg/BW of BaP for 4 days, starting on gestation day 14. Offspring from both BaP-treated and control dams were then sacrificed on post natal day (P) 0, 3, 5, 7, 9,11 and 53. Blood, heart and aorta were harvested upon sacrifice and stored in liquid nitrogen. Microarray and quantitative RT-PCR analysis of the heart and aorta was done to determine the effect of prenatal BaP exposure on gene expression. In addition, western blot analysis was done to determine the effect of BaP on the Sp transcription factors during the development of the rat offspring. Microarray and qRT-PCR data show a number of genes to be significantly altered, while there was a leftward shift in the developmental expression of Sp3. These findings suggest that prenatal BaP exposure affects the normal development of the cardiovascular system of LEH rat offspring.

PS 2204 MODULATION OF OXIDATIVE DNA DAMAGE BIOMARKER IN POSTMENOPAUSAL WOMEN BY GREEN TEA POLYPHENOLS AND TAI CHI.

F. Wang[1], L. Tang[1], G. Qian[1], S. Xue[1], C. L. Shen[2] and J. Wang[1]. [1]*Environmental Health Science, University of Georgia, Athens, GA and* [2]*Texas Tech University Health Science Center, Lubbock, TX.*

Osteoporosis is a degenerative bone disease characterized by low bone mass and structural deterioration of bone tissue and postmenopausal women are high-risk population. Etiological agents associated with osteoporosis include poor nutrition, cytokines, hormones, and aging. Reactive oxygen species (ROS) are shown to be responsible for the aging process and contribute to osteoporosis. Green tea polyphenols (GTP) are capable of reducing the levels of ROS in human studies. On the other side, Tai Cai (TC) exercise was shown to benefit osteoporosis by reducing bone loss. However, the information on effects of GTP and TC, especially their combined effects, on postmenopausal women is lacking. In this study, a total 160 postmenopausal women were recruited to participate in a 6-month placebo-controlled randomized trial. Four treatment arms included placebo, GTP (500mg daily), placebo+TC and GTP+TC. Blood and urine samples are collected at baseline, 1, 3 and 6 month after intervention for analysis of 8-hydroxyldeoxyguanosine (8-OHdG) and GTPs by HPLC-Coularray. The average baseline levels of urinary 8-OHdG are comparable among 4 treatment arms (p=0.299). After 1-month intervention, though no statistical significance among groups, the median level of 8-OHdG decreased 43.9, 27.4, and 40.1% in GTP, TC, and GTP+TC groups, respectively. After 3-month intervention, the median level of 8-OHdG further decreased 62.4, 44.1, and 64.8% in GTP, TC, and GTP+TC groups, respectively (p<0.001). After 6-month intervention, the median level of 8-OHdG decreased 60.1, 40.1, and 73.3% in GTP, TC, and GTP+TC groups, respectively (p<0.001). Serum and urinary levels of GTP components remained constant in GTP and GTP + TC groups at 1, 3 and 6 months, indicating good compliance and pharmacokinetic properties of GTP in the human body. In conclusion, GTP and TC interventions are capable of reducing oxidative DNA damage biomarker levels in postmenopausal women, which should benefit their bone health. (Supported by NCCAM AT003735 and NCI CA 90997).

PS 2205 ANTI-INFLAMMATORY EFFECTS OF FRAGMENTS OF H2A HISTONE AND ORYZA SATIVA JAPONICA PROTEIN.

U. Wormser[1], M. Aschner[2], B. Brodsky[1], E. Proscura[1], A. Erlanger-Rosengarten[1], R. Feldman[1], E. Shapira[1] and Y. Schussheim[1]. [1]*Institute of Drug Research, The Hebrew University, Jerusalem, Israel and* [2]*Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN.*

Anti-inflammatory drugs are often of limited use due to low efficacy and toxic effects. The present study describes the anti-inflammatory effects of a novel nonapeptide termed IIIM1, using the mouse hind paw edema as an experimental model of inflammation. Multiple prophylactic injections of IIIM1 resulted in a significant reduction in carrageenan-induced foot pad swelling, both in mice and rats. A single prophylactic treatment of the peptide caused the maximal effect at 7 to 9 days between the initial peptide treatment and the subsequent carrageenan injection. A reduced inflammatory reaction was observed in transgenic mice constitutively expressing the peptide. A marked decrease in oxidative burst was observed in activated peritoneal macrophages obtained from peptide-treated mice. Furthermore, the sera of IIIM1-treated mice caused a significant decrease in the oxidative burst of macrophages. In addition, the reduction of hind paw swelling in mice injected with the sera of IIIM1-treated mice strongly suggests the presence of a circulating inducible factor responsible for the anti-inflammatory effect of the peptide. Previous LC/MS/MS analysis revealed the presence of a new peptide, termed RA1, in the sera of IIIM1-treated mice. RA1 was identified as a fragment of the Oryza Sativa Japonica protein. The anti-inflammatory effect of RA1 as evidenced by the reduction in carrageenan-induced hind paw swelling corresponded with the decrease in the oxidative burst of macrophages treated in vitro with this peptide. In conclusion, both IIIM1 and RA1 represent potential agents for the efficient treatment of inflammatory diseases that are currently incurable using presently available drugs.
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PS 2206 THE ANALGESIC AND ANTI-PLASMODIAL ACTIVITIES AND TOXICOLOGY OF *VERNONIA AMYGDALINA.*

A. A. Njan[1, 2], B. Adzu[5], A. G. Agaba[2], D. Byarugaba[4], S. Diaz-Llera[3] and D. R. Bangsberg[6]. [1]*Research, Aiida4 Medical Consultants, Abuja, FCT, Nigeria,* [2]*Pharmacology and Therapeutics, Mbarara University of Science and Technology, Mbarara, Uganda,* [3]*Pharmacy, Mbarara University of Science and Technology, Mbarara, Uganda,* [4]*Biology, Mbarara University of Science and Technology, Mbarara, Uganda,* [5]*Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria and* [6]*University of California San Francisco, San Francisco, CA.* Sponsor: S. Areola.

Vernonia amygdalina possesses several bioactive compounds and is used in traditional medicines of south-western Uganda, along with other regions. Its analgesic potential has not been investigated thus far. The present study examines the antinociceptive potential of the aqueous leaf extract (50-200 mg/kg) using three models of nociception (acetic acid-induced writhing, formalin test and tail-flick test), antiplasmodial activity, and toxicology of the extract. The results show the extract significantly inhibits acetic acid-induced writhing and the formalin test in mice but did not give a potent effect in the tail-flick test, suggesting that the extract may have peripheral and central analgesic properties. The extract also inhibited significant antiplasmodial activity in mice against Plasmodium bergheri with 73% inhibition in the group that received a dose of 200 mg/kg i.p. daily for 4 days. Toxicology results show no clinical signs of toxicity or adverse toxicological effects in the treated groups, except for a significant decrease in red blood cell count and a dose of 500-2,000 mg/kg/day for 14 consecutive days as compared to the control. This study supports the traditional use of V. amygdalina as an alternative therapy for malaria and the symptomatic relief of pain usually associated with malaria.

PS 2207 *PLATYCODON GRANDIFLORUM* ROOT-DERIVED SAPONINS SUPPRESSES DNCB-INDUCED ATOPIC DERMATITIS IN MICE.

B. Park[1], J. Choi[1], E. Han[1], Y. Hwang[1], Y. Chung[2] and H. Jeong[1]. [1]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea and* [2]*Food Science, Korea International University, Jinju, Republic of Korea.*

Atopic dermatitis (AD) is a common chronic inflammatory skin disease with various factors, including immunologic abnormalities and exposure to allergens that contribute to the pathogenesis and development of skin lesions. AD has been known to be related with local and systemic immunologic dysfunction that leads to Th1/Th2 response as well as being related to environmental factors. In this study, we examined the preventive effects of 1-chloro-2,4-dinitrobenzene (DNCB)-induced AD by Platycodon grandiflorum root-derived saponins (Changkil saponins: CKS) in NC/Nga mice. NC/Nga mice increase serum IgE levels and severity of the skin lesions under conventional conditions. CKS was administered orally to Nc/Nga mice, which led to the remarkable prevention of the development of AD. CKS reduced DNCB-elevated ear thickness and elevated DNCB-reduced body weight. CKS reduced DNCB-elevated histopathological parameters of AD into the ear. Also, CKS reduced DNCB-elevated serum levels of IgE and thymus- and activation-regulated chemokine, elevated DNCB-reduced serum IL-10 level. These results suggest that CKS prevents the development of DNCB-elevated AD-like skin lesions in Nc/Nga mice.

PS 2208 SUPPRESSION OF PMA-INDUCED TUMOR CELL INVASION BY PIPERINE *VIA* INHIBITION OF PKCα/ERK AND NF-κB/AP-1-DEPENDENT MECHANISMS.

H. Yun[1, 2], Y. Hwang[1], J. Choi[1, 2] and H. Jeong[1]. [1]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea and* [2]*Pharmacy, Chosun University, Gwangju, Republic of Korea.*

In recent years, considerable emphasis has been given to identify new anti-can agents from the natural sources, which could be useful for the human being. Piperine, a major component of black pepper, is used as spice and nutrient enhancer. Several previous studies reported that piperine possesses various beneficial biological activities including antioxidant, anti-atherosclerosis, anti-inflammation, and antiarthritic properties. Although various bioactivity studies of acteoside have been performed, the molecular mechanisms by which the expression of matrix metalloproteinase-9 (MMP-9) and the invasiveness of HT-1080 cells are regulated via acteoside remain unclear. In this study, we examined the inhibitory effect of piperine on phorbol myristate acetate (PMA)-induced MMP-9 expression in HT-1080 human fibrosarcoma cells. Piperine reduced PMA-induced activation of MMP-9 and further inhibited cell invasion and migration. Piperine suppressed PMA-enhanced expression of MMP-9 protein, mRNA and transcription activity levels through suppression of NF-κB and AP-1 activation. Piperine also reduced PMA-enhanced MMP-9 expression through suppression of membrane-type 1 MMP (MT1-MMP), but did not alter tissue inhibitor of metalloproteinase (TIMPs) levels. In addition, piperine suppressed PMA-induced phosphorylation of PKCα and ERK, upstream factors involved in NF-kB and AP-1 pathways. In conclusion, we demonstrated that the anti-invasive effects of piperine might occur through inhibition of PKCα and ERK1/2 phosphorylation and reduction of NF-kB/AP-1 activation, leading to downregulation of MMP-9 expression. Therefore, the inhibition of MMP-9 expression by piperine might have therapeutic potential for controlling the growth and invasiveness of cancer cells.

PS 2209 INHIBITION OF DEVELOPMENT OF ATOPIC DERMATITIS BY SAPONIN FROM THE ROOTS OF *PLATYCODON GRANDIFLORUM.*

E. Han[1], J. Choi[1], J. Im[1], J. Yang[1], Y. Chung[2] and H. Jeong[1]. [1]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea and* [2]*Food Science, Korea International University, Jinju, Republic of Korea.*

Changkil (CK), the aqueous extract of the roots of Platycodon grandiflorum, has been used as a traditional oriental medicine for the treatment of chronic adult diseases. Although a saponin fraction derived from CK (CKS) has been suggested to have a variety of functional effects, its effect on atopic dermatitis is unknown. In this study, we investigated the protective effects of CKS on the development of atopic dermatitis. CKS was administered orally to Nc/Nga mice, which led to the remarkable suppression of the development of dermatitis by dinitrochlorobenzene. Additionally, CKS inhibited the production of thymus- and activation-regulated chemokine (TARC) and its mRNA expression in a keratinocyte cell line, HaCaT, which had been stimulated with tumor necrosis factor-alpha (TNF-a) and interferon-g (IFN-g). Moreover, CKS suppressed NF-kB activation, which may be essential for TARC expression in TNF-a/IFN-g treated HaCaT cells. The inhibitory effect of CKS on NF-kB activation and TARC production might be associated with the anti-atopic dermatitis effects of CKS.

PS 2210 CAPSAICIN SUPPRESSES FIBROSARCOMA CELL MIGRATION AND INVASION *VIA* EGFR-ERK AND P38 MAPK-AP-1 SIGNALING.

H. Jeong, Y. Hwang, J. Choi and B. Park. *Pharmacy, Chungnam National University, Daejeon, Republic of Korea.*

Capsaicin, a pungent ingredient of red pepper, has been reported to possess antitumor activities. The purpose of this study was to investigate the effects of capsaicin treatment on the migration characteristics of human fibrosarcoma HT-1080 cells. Capsaicin inhibited EGF-induced cell migration and invasion. Capsaicin decreased EGF-induced secretion of matrix metalloproteinase (MMP)-9, -2 and membrane-type 1 MMP (MT1-MMP), but did not alter tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 levels in a concentration-dependent manner. In addition, capsaicin decreased EGF-induced activator protein (AP)-1 binding activity and nuclear translocation of c-Jun and c-Fos. Furthermore, EGF-induced phosphorylation of c-Jun, extracellular signal-regulated kinase (ERK)1/2 and p38 mitogen-activated protein kinase (MAPK) were decreased after capsaicin treatment. However, phosphorylation of Jun N-terminal kinase (JNK) was not altered. The EGFR inhibitor AG1478 inhibited EGF-induced secretion of MMP-9, as well as AP-1 activity and cell migration. Collectively, our results suggest that capsaicin inhibits the cancer cell migration and invasion via EGFR-ERK and p38 MAPK-AP-1 signaling pathway.

PS 2211 *PLATYCODON GRANDIFLORUM* ROOT-DERIVED SAPONINS SUPPRESSES ACROLEIN-INDUCED MUC5AC PRODUCTION IN A549 CELLS.

C. Ho[1], B. Park[1], E. Han[1], H. Kim[1], Y. Hwang[1], Y. Chung[2] and H. Jeong[1]. [1]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea and* [2]*Food Science, Korea International University, Jinju, Republic of Korea.*

Mucus hypersecretion contributes to morbidity and mortality in many obstructive lung diseases. In this study, we examined the preventive effects of acrolein-induced MUC5AC production by Platycodon grandiflorum root-derived saponins (Changkil saponins: CKS) in A549 cells. Acrolein increases the MUC5AC which leads to excessive mucus secretion, a hallmark in the pathogenesis of several airway diseases including chronic obstructive pulmonary disease, asthma, and cystic fibrosis. CKS inhibited acrolein-induced MUC5AC mRNA and protein expression. CKS inhibited the acrolein-induced phosphorylation of ERK1/2, JNK1/2 and p38 which is upstream molecules in MUC5AC synthesis. Inhibitors of MAPK, such as ERK inhibitor PD98059, JNK1/2 inhibitor JNK inhibitor II and p38 MAPK inhibitor SB203580, inhibited the acrolein-induced MUC5AC mRNA and protein expression. CKS inhibited the acrolein-induced phosphorylation of PKC delta (PKCσ). Inhibitor of PKCσ, rottlerin, inhibited the acrolein-induced MUC5AC mRNA and protein expression. CKS inhibited the acrolein-induced ROS production. Inhibitor of ROS, N-acetylcysteine, inhibited the acrolein-induced MUC5AC mRNA and protein expression. CKS inhibited the acrolein-induced NF-kB activity. Inhibitor of NF-kB, NF-kB activation inhibitor, inhibited the acrolein-induced MUC5AC mRNA and protein expression. Also, CKS inhibited the acrolein-induced NF-kB nuclear translocation and NF-kB p65 phosphorylation. CKS inhibited the acrolein-induced IkBα degradation. These results suggest that preventive effect of CKS is mediated through the inhibition of MUC5AC expression by the inhibition of NF-kB via activation of MAPKs pathways with PKCα and ROS.

PS 2212 ANTHOCYANIN ISOLATED FROM THE PURPLE-FLESHED SWEET POTATO PREVENTS HEPATIC FIBROSIS BY DMN AND HEPATIC STELLATE CELLS PROLIFERATION BY PDGF-BB.

J. Choi[1], Y. Hwang[1], C. Choi[2], Y. Chung[2] and H. Jeong[1]. [1]*Pharmacy, Chungnam National University, Daejeon, Republic of Korea and* [2]*Food Science, Korea International University, Jinju, Republic of Korea.*

Hepatic fibrosis and its end-stage disease, cirrhosis, is one of the world's major health problems. During the development of hepatic fibrosis, activated hepatic stellate cells (HSCs) are ability to synthesize and deposit excessive extracellular matrix

components into the liver parenchymal cell. In this study, we investigated the preventive effects of the anthocyanin fraction (AF) isolated from the purple-fleshed sweet potato on dimethylnitrosamine (DMN)-induced hepatic fibrosis and on platelet-derived growth factor (PDGF)-BB-induced HSCs proliferation. AF inhibited DMN-induced serum alanine aminotransferase and aspartate aminotransferase activities and elevated DMN-reduced body and liver weights. AF inhibited DMN-induced histopathological changes and collagen contents. Also, AF inhibited DMN-induced expression of alpha-smooth muscle actin (α-SMA), type I and III collagen, PDGF receptor-beta (PDGFR-β), tumor necrosis factor-α and transforming growth factor-βl. The increased number of HSCs during liver fibrogenesis reflects the activity of several growth factors and their cognate tyrosine kinase receptors. AF suppressed HSCs activation, including PDGF-BB-induced proliferation as well as α-SMA expression by PDGF-BB-induced Akt and ERK1/2 phosphorylation. Also, AF suppressed the phosphorylation of PDGFR-β following PDGF-BB stimulation, providing a mechanism for the inhibition of AF-mediated tyrosine kinase. These results suggest that AF exhibits anti-fibrotic and anti-proliferation properties, may be of potential therapeutic value in protecting the development of chronic liver disease.

PS 2213 *PLATYCODON GRANDIFLORUM* ROOT-DERIVED SAPONINS SUPPRESSES HIGH FAT DIET-INDUCED STEATOSIS AND FIBROSIS IN RATS.

Y. Chung[2], J. Choi[1], T. Khanal[1], B. Park[1], Y. Hwang[1] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea and [2]Food Science, Korea International University, Jinju, Republic of Korea.*

Nonalcoholic fatty liver disease, induced by nutritional factors, is one of the leading causes of hepatic dysfunction in the world. The activation of inflammatory signaling in the liver, which is induced by systemic and locally produced cytokines, and the development of hepatic factors associated with the progression to steatosis, a pre-cirrhotic condition. In this study, we examined the preventive effects of high fat diet (HFD)-induced hepatic steatosis, inflammation and fibrosis by Platycodon grandiflorum root-derived saponins (Changkil saponins: CKS) in rats. CKS reduced HFD-elevated serum alanine aminotransferase and aspartate aminotransferase activities, and body and liver weights. CKS reduced HFD-elevated histopathological changes of the hepatic steatosis, inflammation, ballooning degeneration and fibrosis. CKS reduced HFD-elevated hepatic levels of triglycerol, total cholesterol and low density lipoprotein cholesterol. CKS elevated HFD-reduced hepatic level of high density lipoprotein cholesterol. Furthermore, CKS reduced HFD-elevated expression of sterol regulatory element binding protein-1, and elevated HFD-reduced expression of fatty acid synthetase and silent information regulator 1 and phosphorylation of acetyl-CoA carboxylase and AMP-activated protein kinase. Also, CKS reduced HFD-elevated expression of cyclooxygenase-2, alpha-smooth muscle actin, type I and III collagen and collagen contents. CKS reduced the HFD-elevated NF-kB nuclear translocation and IkBα degradation. These results suggest that CKS might be a critical preventive part in response to inflammation, steatosis, and fibrogenesis in the liver.

PS 2214 ACTEOSIDE SUPPRESSES PHORBOL 12-MYRISTATE 13-ACETATE-INDUCED MMP-9 EXPRESSIONS *VIA* CAMK/ERK- AND JNK/NF-KB SIGNALING PATHWAY IN HUMAN FIBROSARCOMA HT-1080 CELLS.

Y. Hwang, J. Choi, E. Han, H. Kim, B. Park and H. Jeong. *Pharmacy, Chungnam National University, Daejeon, Republic of Korea.*

Several studies have suggested that intake of food rich in antioxidants is associated with a lower risk of some chronic diseases. Acteoside is a well-studied phenylethanoid glycoside that is widely distributed in the plant kingdom. Several studies have indicated that acteoside exhibits anti-oxidative, anti-inflammatory, and neuronal protective activities. Although various bioactivity studies of acteoside have been performed, the molecular mechanisms by which the expression of matrix metalloproteinase-9 (MMP-9) and the invasiveness of HT-1080 cells are regulated via acteoside remain unclear. In this study, we examined the effects of acteoside on PMA-induced MMP-9, and explored the underlying upstream molecular signaling events involved in this regulation. We found that acteoside suppresses PMA-enhanced MMP-9 expression at the protein, mRNA, and transcriptional levels through the suppression of NF-kB activation. In addition, acteoside repressed the PMA-induced phosphorylation of ERK1/2 and JNK1/2. Further, we found that acteoside decreased the PMA-induced influx of Ca2+ and repressed PMA-induced CaMK phosphorylation. Furthermore, treatment with BAPTA/AM, W7, or capsazepine markedly decreased PMA-induced MMP-9 secretion and cell migration, as well as ERK and JNK/NF-kB activation. In summary, the present data demonstrate that acteoside inhibits the PMA-induced invasion and migration of human fibrosarcoma cells via Ca2+-dependent CaMK, ERK, and JNK/NF-kB signaling.

PS 2215 AMP-ACTIVATED PROTEIN KINASE IS REQUIRED FOR THE LIPID-LOWERING EFFECT OF ANTHOCYANINS-RICH PURPLE SWEET POTATO IN INSULIN-RESISTANT HUMAN HEPG2 CELLS.

J. Kim, Y. Hwang, J. Choi and H. Jeong. *Pharmacy, Chungnam National University, Daejeon, Republic of Korea.*

AMP activated protein kinase (AMPK), an energy sensor, has extensive regulatory effects on lipid and carbohydrate metabolism. The activation of AMPK protects against fatty acid synthase (FAS) induction and lipid accumulation caused by high glucose. Anthocyanins of the purple sweet potato exhibit antioxidant, hepatoprotective, antiobesity and antihyperglycemic activities. In this study, we investigated that the effect of anthocyanin fraction (AF) obtained from the purple sweet potato on high glucose-induced lipid accumulation and FAS induction. In HepG2 hepatocytes, AF increased phosphorylation of AMPK and its downstream target, acetyl-CoA carboxylase (ACC). AF also prevented the lipid accumulation and FAS expression that occurred in HepG2 cells exposed to high glucose. In conclusion, AF lowers hepatic lipid content and FAS expression by activating AMPK, thereby mediating beneficial effects in hyperglycemia and insulin resistance.

PS 2216 ANTHOCYANINS FROM PURPLE SWEET POTATO PROTECTS AGAINST TERT-BUTYL HYDROPEROXIDE-INDUCED OXIDATIVE STRESS BY INCREASING EXPRESSION OF HEME OXYGENASE-1 IN A PI3K AND ERK/NRF2-DEPENDENT MANNER.

H. Pil[1], J. Choi[1], J. Seo[2], Y. Chung[2] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea and [2]Food Science, Korea International University, Jinju, Republic of Korea.*

Anthocyanins of purple sweet potato possess biological functions such as scavenging free radicals, anti-mutagenicity, anti-carcinogen activity and anti-hypertensive effects. However, the signaling pathways involved in the actions of anthocyanins-induced antioxidant enzymes against cytotoxicity are not fully understood. In current study, we investigated the protective effects of an anthocyanin fraction (AF) obtained from purple-fleshed sweet potato against tert-butyl hydroperoxide (t-BHP)-induced cytotoxicity in HepG2 cells. Pretreatment of HepG2 cells with AF significantly reduced t-BHP-induced generation of ROS, caspase-3 activation, and subsequent cell death. Also, AF up-regulated heme oxygenase-1 (HO-1) expression, which conferred cytoprotection against oxidative in¬jury induced by t-BHP. Moreover, AF in¬duced nuclear translocation of the transcription factor NF-E2-related factor 2 (Nrf2), which is upstream of AF-induced HO-1 expression, and PI3K/Akt and ERK1/2 activation, a pathway that is involved in induced Nrf2 nuclear translocation, HO-1 expression and cytoprotection. The present results suggest that the protective effects of AF against t-BHP-induced cytotoxicity may be due, at least in part, to its ability to scavenge ROS and to regulate the antioxidant enzyme HO-1 via the PI3K/Akt and ERKl/2-Nrf2 signaling pathways.

PS 2217 SAPONINS FROM THE ROOTS OF *PLATYCODON GRANDIFLORUM* STIMULATE OSTEOBLAST DIFFERENTIATION *VIA* RUNX2 ACTIVATION IN MESENCHYMAL STEM CELL.

H. Jeong[2], J. Kim[1], E. Han[1], B. Park[1], Y. Chung[3], K. Lee[2] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea, [2]Pharmacy, Chonnam National University, Gwangju, Republic of Korea and [3]Food Science, Korea International University, Jinju, Republic of Korea.*

Changkil (CK), the aqueous extract of the roots of Platycodon grandiflorum, has been used as an oriental medicine for the treatment of chronic diseases. In the present study, the effects of saponin fraction derived from CK (CKS) on osteoblast differentiation and function were determined by analyzing the activity of alkaline phosphatase (ALP), an osteoblast marker, and the regulation of RUNX2, a master gene of osteoblast differentiation, in a mesenchymal stem cell line C2C12 cells. CKS induced ALP activity and the expression of osteogenic marker genes in C2C12 cells. Additionally, CKS increased the expression and transcriptional activity of RUNX2. To determine which signaling pathways are involved in the osteogenic effects of CKS, we tested the effect of inhibitors of kinases known to regulate RUNX2. These results suggest that CKS stimulates osteoblast differentiation by activation of RUNX2 and the regulation of RUNX2 activation by CKS may be an important therapeutic target for osteoporosis.

PS 2218 *PSIDIUM GUAJAVA* INHIBITS IGE-MEDIATED ALLERGIC RESPONSES BY BLOCKING FCεRI SIGNALING IN MAST CELLS.

J. Im[1], E. Han[1], J. Park[1], J. Yang[1], J. Seo[2], Y. Chung[2] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea and [2]Food Science, Korea International University, Jinju, Republic of Korea.*

Psidium guajava (P. guajava) is a food and plant with antioxidant, anti-inflammatory, and anti-allergic activities, supporting its traditional uses. However, its precise effects remain unknown. We investigated the effects of P. guajava ethyl acetate extract (PGEA) on allergic responses in rat mast RBL-2H3 cells. PGEA reduced DNP-BSA (antigen)-induced release of β-hexosaminidase and histamine in IgE-sensitized mast cells. Additionally, it inhibited antigen-induced IL-4 and TNF-α mRNA expression and protein production in IgE-sensitized mast cells. PGEA also reduced antigen-induced COX-2 mRNA and protein expression in these cells. Moreover, it inhibited antigen-induced activation of NF-κB and degradation of IκB-α. To identify the mechanisms underpinning the inhibition of degranulation and cytokine production by PGEA, we examined the activation of intracellular FcεRI signaling molecules. PGEA suppressed antigen-induced phosphorylation of Syk, LAT, Gab2, and PLCγ2 but not Lyn. Collectively, the anti-allergic effects of PGEA in vitro suggest its possible therapeutic application to inflammatory allergic diseases, in which its inhibition of inflammatory cytokine production and FcεRI-dependent signaling events in mast cells may be hugely beneficial.

PS 2219 NOVEL PHARMACOLOGICAL ACTIONS OF NATURAL ANTAGONISTS DERIVED FROM *K. BREVIS* (RED TIDE).

W. M. Abraham[1] and D. G. Baden[2]. *[1]Research, Mount Sinai Medical Center, Miami Beach, FL and [2]Marine Science, University of North Carolina Wilmington, Wilmington, NC.*

Brevenal and B-naphthoyl brevetoxin (B-Nap) are two antagonists derived from Florida red tide organisms. We previously reported that aerosol treatment with pMolar concentrations of these agents a) improves mucociliary clearance (MCC) and b) prevents and reverses human neutrophil elastase (HNE)-induced slowing of MCC, responses not seen with inhaled glucocorticosteroids. These actions suggested that brevenal and B-Nap are potential drug candidates for the treatment of diseases associated with MCC dysfunction. We extended our safety assessment of these compounds by defining maximal aerosol doses that could be delivered without causing bronchoconstriction (< 100 ug/ml) and showing that repeat dosing (b.i.d. 50 ug/ml for 5 days) does not cause airway hyperresponsiveness. Thus, neither agent induces airway irritant effects at doses 50,000-fold higher than needed to show pharmacological activity. Here we describe novel actions of both brevenal and B-Nap, i.e. blocking histamine- and HNE-induced bronchoconstriction, which increases their potential for drug development. Increasing concentrations of aerosol histamine were given to sheep to generate histamine dose response curves. Brevenal and B-Nap (20 breaths of 100 pg/ml) given 30 min before histamine challenge reduced the histamine response by 72 and 68%, respectively. These results compared favorably with the clinical drug clarinex (5 mg, PO) which gave 67% protection. Extending the pretreatment time to 2h before histamine challenge gave 36% and 59% protection with brevenal and B-Nap, respectively. The same dose of these agents given 30 min before HNE-challenge inhibited the constrictor response by 66%. These results indicate that these naturally-derived marine products show pharmacological activity against prominent mediators associated with airway allergic and inflammatory responses. Their protective actions are multifaceted and are seen at nMolar concentrations. Both characteristics are advantageous: broad spectrum protection with a potential reduction in higher dose-induced side effect profile.

PS 2220 BIOACTIVATION EVALUATION OF HERBAL INGREDIENTS.

Z. Fang, Y. Zhang, H. Ma, G. Li and L. Yang. *Laboratory of Pharmaceutical Resource Discovery, Dalian Institute of Chemical Physics, Chinese Academy of Scienes, Dalian, Liaoning, China.* Sponsor: P. Wells.

Elucidation of toxicological properties of herbal medicines is becoming more and more important with the increasing application of herbal medicines for treatment of various diseases and promotion of health. It has been widely recognized that bioactivation of herbal components represents a critical reason for herbal toxicity induction including direct herbal toxicity and herb-drug interaction. To date, over 20 "structural alerts" have been reported to easily undergo bioactivation and induce toxicity. Among these toxicophores, methylenedioxyphenyl and indole groups were often found in natural products. Therefore, the focus was given to bioactivation evaluation of these two classes of herbal constituents in the present study. Noscapine and rutaecarpine were selected as model compounds. In vitro chemical trapping method was adapted. The experiment was carried out in human liver microsomes with NADPH-generating system, reduced glutathione (GSH) and tested compound. The adducts were detected using LC-MS/MS. Chemical inhibition study and recombinant CYP isoforms assay were employed to identify the enzymes involved in the formation of reactive metabolites. The results showed that the corresponding GSH conjugate was formed, and ortho-quinone and quinone-imine metabolites were proposed to be reactive intermediates of noscapine and rutaecarpine, respectively. Furthermore, CYP3A4, CYP2C9 were major responsible CYP isoforms for noscapine's bioactivation, and CYP3A4, CYP1A2 were mainly involved in the bioactivation of rutaecarpine. All these experiments demonstrated that potential risk associated with herbal bioactivation might exist when these natural products were clinically used. However, given that many other factors (e.g. herbal complexity, variability of biological systems, etc.) might influence the explanations of herbal bioactivation, further experiments need for the toxicity evaluation of these natural products.

PS 2221 CYTOTOXIC EFFECTS OF ARBUTIN IN MAMMALIAN CELLS AFTER ACTIVATION BY HUMAN INTESTINAL BACTERIA.

H. Kim[1], T. Khanal[1], H. Ha[2], T. Jeong[2] and H. Jeong[1]. *[1]Pharmacy, Chungnam National University, Daejeon, Republic of Korea and [2]Pharmacy, Yeungnam National University, Gyeonsgan, Republic of Korea.*

Hydroquinone is present in a number of plant-derived foods such as wine, coffee, broccoli and certain fruits, where it predominantly occurs in its glycosylated form: Arbutin (hydroquinone-beta-D-glucopyranoside). This study was conducted to investigate the cytotoxic responses of arbutin and its metabolites (hydroquinone) to determine the metabolism of cytotoxic activities. Hydroquinone, arbutin metabolites, increases strong cytotoxic effect in hepatoma cells, but not with arbutin. In addition, hydroquinone-induced apoptosis was confirmed by apoptosis assays. Furthermore, increased ROS generation was noted by the treatment of hydroquinone to compare with arbutin. Following an incubation of arbutin with intestinal bacteria for 24 hr, it was detected that arbutin was metabolized to hydroquinone. Arbutin metabolites showed more potent cytotoxicity against mammalian cells than arbutin control. These findings suggest that intestinal microflora may activate the cytotoxic effect of metabolism of arbutin to hydroquinone in hepatoma cells. [This research was supported by a grant (09172KFDA996) from Korea Food & Drug Administration in 2010.]

PS 2222 PREVENTION OF ADRIAMYCIN HEPATIC AND RENAL TOXICITY IN MALE BALB/C MICE BY A NUTRIENT MIXTURE.

M. Roomi, N. W. Roomi, M. Rath and A. Niedzwiecki. *Dr. Rath Research Institute, Santa Clara, CA.*

Introduction: Adriamycin (ADR) or doxorubicin is an antineoplastic antibiotic used in cancer therapy. However, several studies suggest that its increasing acute and chronic use is associated with toxicity of vital organs. A unique nutrient mixture (NM) consisting of lysine, proline, ascorbic acid and green tea extract has previously been shown to exhibit a broad spectrum of pharmacological, therapeutic, cardiovascular and chemopreventive properties. Objective: This study was undertaken to determine whether NM is useful in prevention of ADR-induced hepatic and nephric toxicity. Materials and Methods: Six week-old male BALB/c mice were divided into four groups (A-D) of six animals each. Groups A and C were fed a regular diet for three weeks, while groups B and D were fed a diet supplemented with 1% NM during that period. After three weeks, mice in groups C and D received ADR 20 mg/kg body weight i.p., and those in groups A and B received saline alone. All animals were sacrificed after 24 hrs, blood was withdrawn by cardiac puncture, serum collected for clinical chemistry, and livers, kidneys and hearts were excised for histology. Results: Administration of ADR to group C resulted in marked increase in ALT, AST and gamma-GT levels, whereas in group D (NM diet) the serum ALT, AST and gamma-GT activities were not affected, but were comparable to the levels of the control group A and NM-fed group B. Administration of ADR also resulted in significantly increased levels of serum markers for kidney (BUN, creatinine and uric acid) in group C. In contrast, animals in group D exhibited similar levels to those in groups A and B. The histological changes will be discussed in view of their blood chemistry. Conclusions: The results indicate that NM has potential to protect against ADR-induced hepatic and nephric damage.

PS 2223 ISOLATION AND DETECTION OF RICIN IN CASTOR BEANS USING A DUAL-FRACTIONATION TECHNIQUE AND MATRIX-ASSISTED LASER DESORPTION IONIZATION/TIME-OF-FLIGHT MASS SPECTROMETRY.

C. Wilson, K. Meyerholtz and S. Hooser. *Indiana Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN.*

Toxicoproteomics encompasses the global characterization of proteins that change in cells, tissues, and other biological samples resulting from exposure to a variety of toxicants. While this approach is more commonly used for biomarker profiling, proteomic investigations can also be used for detecting specific protein toxins, such as the glycoprotein ricin, for diagnostic toxicology. This study exploits this aspect by integrating two fractionation techniques, size exclusion and lectin affinity selection, into a proteomics platform designed to isolate and identify ricin in castor beans. This procedure briefly involves the following: 1) Five grams of castor beans were pulverized and delipidated using ethyl ether, 2) castor bean proteins from the water-soluble portion that were greater than 20kDa were selected using size exclusion fractionation, 3) glycoproteins from this fraction were then isolated using concanavalin A lectin affinity selection, 4) the glycoproteins were then digested into peptides using trypsin, and 5) the resultant peptides were detected using matrix-assisted laser desorption ionization/time-of-flight mass spectrometry. The monoisotopic, peptide ions were imported into the ProteinProspector database search engine for protein identification. Mass spectral data revealed several peptide sequences isolated from the castor beans, of which 4 were identified as ricin agglutinin A chain. Due to the inherent proteinaceous nature of most samples, employing this dual-fractionation technique provides a means to selectively isolate ricin from castor beans while simplifying sample complexity for proteomics analysis. Future studies will include using this procedure to isolate and detect ricin in biological samples for diagnostic toxicology cases.

PS 2224 PROTECTIVE EFFECT OF *HOVENIA DULCIS* EXTRACT ON THE TUNICAMYCIN INDUCED ER STRESS IN HEPG2 CELLS.

M. Dong[1], J. Choi[1], C. Na[2], D. Na[1] and Y. Park[1]. [1]*School Lifesciences and Biotechnology, Korea University, Seoul, Republic of Korea and* [2]*Lifetree Biotech Co., Suwon, Republic of Korea.* Sponsor: B. Lee.

Hovenia dulcis, known as Japanese raisin tree, has a long history as a food supplement and traditional medicine in Japan, China and Korea. Extracts from Hovenia dulcis accelerate the detoxification of ethanol, and possess hepatoprotective, antioxidative, and antidiabetic properties. However the underlying molecular mechanisms are not sufficiently understood. In the present study, the protective effect of Hovenia dulcis hot water extract (HWE) on the hepatotoxicity induced by tunicamycin, a representative ER stressor, was observed in HepG2 cells using cytotoxicity, reporter gene assay, ER stress related gene expression. For the reporter gene assay, we prepared the reporter gene, pGL4-Grp78-189-Luc which contained -165 to +23 region of grp78. HepG2 cells were plated into 48 wells, transfected with pGL4-Grp78-189-Luc, pretreated with various concentrations of HWE for 24 h and added tunicamycin (10 μg/ml) for 16 h. Tunicamycin was elevated the activity of luciferase more than 2 folds and HWE was reduced the luciferase activity in a dose dependent manner. Next, we observed the ER stress related gene expressions by mRNA and immunoblotting. Cells were pretreated with Hovenia dulcis extract for 24 h and then ER stress was induced with tunicamycin. HWE was down-regulated the mRNA of CHOP (transcription factor, C/EBP homologous protein), XBP1 (X-box binding protein-1) and GRP78 (78 kDa glucose-regulated protein) expression. CHOP protein level was significantly decreased pretreatment with 25 and 200 μg/ml Hovenia dulcis extract in HepG2 cells. This finding suggested that hepatoprotective effects of Hovenia dulcis extract could be related to attenuation of ER stress by various toxic compounds.

PS 2225 EFFECTS OF THE MARINE ALGAL TOXIN PECTENOTOXIN ON THE ACTIVITY OF VARIOUS ACTIN ISOFORMS.

S. Butler[1], C. O. Miles[2], A. Karim[1] and M. Twiner[1]. [1]*Natural Sciences, University of Michigan, Dearborm, MI and* [2]*Section for Chemistry, National Veterinary Institute, Oslo, Norway.*

Pectenotoxins (PTX) are marine toxins produced by dinoflagellates that accumulate in shellfish, leading to human intoxication. There are at least 15 different analogues of PTX with slight variation in structure leading to different chemical properties and consequently, different *in vivo* toxicities. Since preliminary studies have shown that the parent compound PTX-2 targets actin, we have been studying the effects of three analogs of pectenotoxin, PTX-2, PTX-2 seco acid, and PTX-11, on the polymerization and depolymerization of skeletal muscle actin, smooth muscle actin, cardiac muscle actin, and non-muscle actin. An optimized actin assay based on fluorescently labeled skeletal muscle actin and SDS-PAGE were jointly used to determine the relative amounts of filamentous and globular actin formed during polymerization and depolymerization experiments. Our findings suggest that PTX-2 causes a dose-dependent decrease in both the rate and yield of skeletal muscle actin polymerization (IC_{50}s of 43 and 179 nM; respectively), with no significant effects on depolymerization. Inhibitory effects of PTX-2 seco acid and PTX-11 (up to 500 nM) on actin polymerization were not observed. Future experiments will characterize the effects of PTX on the other actin isoforms using semi-quantitative SDS-PAGE methods.

PS 2226 COMPARATIVE CYTOTOXICITY OF VARIOUS ANALOGS OF THE MARINE ALGAL TOXIN AZASPIRACID.

R. El-Ladki[1], G. Doucette[2] and M. Twiner[1]. [1]*Natural Sciences, University of Michigan, Dearborm, MI and* [2]*Marine Biotoxins Program, NOAA/National Ocean Service, Charleston, SC.*

Azaspiracids (AZA) are polyether marine toxins produced by a small dinoflagellate that accumulates in shellfish and represent an emerging human health risk. Although monitored and regulated in many European and Asian countries, there are no regulatory requirements or standards in the United States for this toxin group. This did not prove to be a problem until June 2009 when AZAs were identified in US seafood for the first time resulting in human intoxications. Efforts are now underway to better define the effects and mechanism(s) of action for the AZAs. Our investigations have employed Jurkat lymphocyte T cells as an *in vitro* cell model to characterize the potential toxicological effects of AZA1, AZA2 and AZA3. Cytotoxicity experiments employing a metabolically-based dye (i.e., MTS) indicated that AZA1, -2, and -3 each exhibited a lethal response that was both concentration- and time- dependent with EC_{50} values in the low or sub-nanomolar range. Based on curve shift analysis and EC50 comparisons, the order of potency was as follows: AZA2 > AZA3 > AZA1 with toxin equivalency factors (TEFs) of 8.3 and 4.5 for AZA2 and AZA3, respectively, relative to AZA1. Image analysis of the cells using Nomarski differential interference contrast (DIC) and cellular actin fluorescence indicate that the morphological effects of AZA1 on this cell type are unique relative to the effects of AZA2 and AZA3. Ongoing experiments continue to investigate the relative TEFs using additional cell lines and to further define AZAs mechanism of action.

PS 2227 ANALYSIS OF RESVERATROL AND ITS METABOLITES IN PREGNANT RATS, FETUSES, AND PUPS.

B. L. Fletcher[1], M. A. Silinski[1], T. R. Fennell[1], F. K. Thomas[1], J. C. Blake[1], S. D. Cooper[1], R. A. Fernando[1] and B. J. Collins[2]. [1]*RTI International, Research Triangle Park, NC and* [2]*NIEHS/NTP, Research Triangle Park, NC.*

Resveratrol (RES) is a naturally-occurring polyphenol found in grapes and red wine that has been reported to have antioxidant and cardioprotective effects. RES undergoes extensive first pass metabolism, but little is known about the kinetics and distribution of RES metabolites. Exposure to RES in diet and supplements has raised concern about potential toxicity. The objective of this work was to develop and apply methods for determination of RES and its metabolites in rat plasma, fetus, and pup tissues to assess perinatal exposure and metabolism of RES. *Trans*-RES was administered orally at 78, 312.5, or 1250 mg/kg to pregnant and lactating Wistar-Han rats from gestation day (GD) 11 through post-natal day (PND) 4. Dam plasma and fetuses were collected on GD18, and pups were collected on PND4. A second set of pups were dosed PND12-21, and plasma was collected on PND21. Plasma samples were extracted with acetonitrile, and fetus and pup homogenates with methanol. UPLC-MS/MS analysis was conducted using electrospray ionization in negative ion mode and multiple reaction monitoring. The method has been successfully applied to the analysis of RES and its metabolites, 3-O-β-D-glucuronide (GLUC) and 3-sulfate (SULF), in plasma, pups, and fetuses. Dam plasma concentrations ranged from below the limit of quantitation (BLOQ, 5.06 ng/mL) to 4590 ng/mL for RES, BLOQ (96.5 ng/mL) to 164,000 ng/mL for GLUC, and BLOQ (5.03 ng/mL) to 94,100 ng/mL for SULF. Significant differences in ratios were observed for the PND21 pups; plasma concentrations ranged from BLOQ to 2070 ng/mL for RES, BLOQ to 508,000 ng/mL for GLUC, and BLOQ to 48,400 ng/mL for SULF. Significant levels of RES, GLUC, and SULF were also observed in the pup and fetal tissues. These results indicate that RES is rapidly and differentially metabolized to GLUC and SULF in dams and pups, and that RES is transferred to the fetus in pregnancy and to the pup by lactation.

PS 2228 AFB1 BINDING CAPACITIES OF EXCELERITE CLAY SAMPLES FROM PANACA, NEVADA.

K. Zychowski[1], A. Marroquin-Cardona[1], Y. Deng[2] and T. D. Phillips[1]. [1]*College of Veterinary Medicine, Texas A&M University, College Station, TX and* [2]*Soil and Crop Sciences, Texas A&M University, College Station, TX.*

Aflatoxin B1 (AFB1) is a mycotoxin mainly produced by the fungi *Aspergillus flavus* and *A. parasiticus*, which commonly contaminate corn, peanut and other various agricultural crops. This toxin is a known cause of aflatoxicosis and an important contributing factor in liver cancer. In previous studies our laboratory has observed, NovaSil (NS), a montmorillonite clay to be an active ingredient for aflatoxin adsorption. It is expected that other montmorillonites besides NS clay will have similar sorption capabilities, especially dioctahedral smectites. For instance, EXCELERITE (U.S. Natural Nutrient and Minerals Inc.) is a clay-based natural product from a unique mine in Panaca, Nevada suspected to contain smectite. Hence, our objectives in the present study were to investigate the mineral characteristics and AFB1 binding potential of EXCELERITE by using X-ray diffraction (XRD) techniques and isothermal adsorption analyses. Samples of EXCELERITE were collected throughout 14 various regions of the mine and analyzed accordingly. Based on Q-max calculations from isothermal data, NS appears to have a higher AFB1 sorption capacity than the samples from the EXCELERITE mine. X-ray diffraction patterns were run on the most promising samples to identify mineral content; revealing the presence of smectite (most likely montmorillonite). The sample with the best sorption characteristics was selected for further analysis, and the clay:silt:sand ratio was determined. Sorption analysis was performed with the less than 2µm component as well as in a combination with NS in a 1:1 ratio. Q-max values for the samples are as follows: NS 0.38, 1:1 mixed sample 0.22, half-NS (50mg) 0.18, <2µm EXCELERITE 0.16. A hydra bioassay was performed as a preliminary test for toxicity, and while some of the hydra had a mild response to the samples, none of hydra resulted in overt toxicity. Further work is warranted to confirm the efficacy of this product for prevention of aflatoxicosis in animals.

PS 2229 THE ACUTE TOXICITY OF THE DEATH CAMAS (*ZIGADENUS SPP.*) ALKALOID ZYGACINE IN MICE, INCLUDING THE EFFECT OF METHYLLYCACONITINE CO-ADMINISTRATION ON ZYGACINE TOXICITY.

K. Welch, K. Panter, D. Gardner, B. Stegelmeier, B. Green, J. Pfister and D. Cook. *Poisonous Plant Research Laboratory, USDA-ARS, Logan, UT.*

Death camas (*Zigadenus spp.*) is a common poisonous plant on foothill rangelands in western North America. The steroidal alkaloid zygacine is believed to be the primary toxic component in death camas. In most cases where livestock are poisoned by plants in a range setting, there is more than one potential poisonous plant in that area. One common poisonous plant that is often found growing simultaneously in the same area as death camas is low larkspur (*Delphinium nuttallianum*). The objective of this study was to determine if co-administration of low larkspur will exacerbate the toxicity of death camas. We first characterized a number of different parameters describing the acute toxicity of zygacine in mice. The i.v. LD50 of total alkaloid extracts from a Utah and a Nevada collection were 2.8 ± 0.8 and 2.2 ± 0.3 mg/kg, respectively. The LD50 of zygacine administered i.v. and orally was 2.0 ± 0.2 mg/kg and 132 ± 21 mg/kg, respectively. The elimination half-life of zygacine from whole blood was determined to be 13.0 ± 2.7 min. The i.v. LD50 of methyllycaconitine (MLA), one of the major toxic alkaloids in low larkspur, was 4.6 ± 0.5 mg/kg. In comparison, the i.v. LD50 of a 1:1 mixture of MLA and zygacine was 2.9 ± 0.7 mg/kg. The clinical signs in mice treated with this mixture were very similar to those of mice treated with zygacine alone, including the time of onset and death. These results suggest that there is not a synergistic effect of co-administering these two alkaloids intravenously in mice, however, there appears to be an additive effect. The results from this study increase knowledge and understanding regarding the acute toxicity of death camas. As combined intoxications are most likely common, this information will be useful in developing management recommendations for ranchers and in designing additional experiments to study the toxicity of death camas to livestock.

PS 2230 TOXICOLOGICAL ASSESSMENT OF METHANOLIC ROOT EXTRACT OF *CNESTIS FERRUGINEA*: CHARACTERIZATION OF ANTIOXIDANT ENZYME ACTIVITY IN SPRAGUE-DAWLEY RAT.

I. Ishola, J. Akindele and O. Adeyemi. *Pharmacology, University of Lagos, Lagos, Lagos, Nigeria.*

The methanolic root extract of *Cnestis ferruginea* (CF) Vahl ex DC (Connaraceae) is widely employed in the treatment of various ailment in traditional medicine throughout West Africa. Due to long term uses, this study was carried out to evaluate the subchronic toxicity patterns of the plant. The 90 days subchronic studies were conducted in rats with oral daily doses of 80, 400 and 1000mg/kg of CF which was followed by 14 days reversibility study without treatment. Daily oral doses induced no significant change in body weight and vital organ weight ($p>0.05$). There was statistically significant decrease in the weight of the testis at 1000mg/kg ($p<0.05$) but no significant change in ovary weight. No lethality was observed. However, haematological and biochemical parameters showed a significant decrease in platelet count (1000mg/kg) ($p<0.05$) and an increase in AST/ALT ratio in a dose dependent manner but not significant ($p>0.05$) and an increase in uric acid levels. There was significant decline (40-70%) in epididymal sperm count and 33-60% in sperm motility as well as substantial abnormalities in sperm morphology. Significant variances in activities of antioxidant enzymes were also observed; increase in testes and brain MDA level with a decrease in testes and brain GSH level in male treated but an increase in female rats. However, there were significant increases in liver, kidney and ovary GSH, SOD and Catalase activities, in addition, a significant dose dependent decrease in MDA level ($p<0.05$, $p<0.01$). The histological studies showed normal architecture of the liver, kidney, heart, testes and ovary. After the 14 days reversibility studies, there was significant ($p<0.05$) reversal in the serum levels of the biochemical and haematological parameters investigated. These result showed that chronic consumption of CF induced spermatogenic damage, as indicated by a decrease in sperm count and motility, also a decrease in testicular antioxidant enzymes.

PS 2231 POMEGRANATE EXTRACT EXERTED CHEMOPREVENTIVE EFFECTS IN COLON CANCER CELLS AND TARGETED MICRORNA-21A AND MICRORNA-126.

N. Banerjee[1], G. Noratto[1], L. Xiangrong[1] and S. M. Talcott[1, 2]. [1]*Nutrition and Food Science, Texas A&M University, College Station, TX and* [2]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.*

The anti-cancer efficacy of polyphenols extracted from pomegranate (Punica granatum L.) has been extensively studied; however the involvement of microRNAs in underlying mechanisms is still unknown. The objective of the present study was to investigate the role of post-transcriptional regulation of apoptotic and inflammatory biomarkers by specific microRNAs in pomegranate extract (Pg) treated colon cancer cells. Cell growth inhibition was assessed by cell counting and mRNA and microRNA expression by RT-PCR and protein expression by western blotting. MicroRNAs were analyzed by RT-PCR and transfection with dual-luciferase reporter plasmids containing the respective target sequence for the tested microRNAs. The involvement of microRNAs was confirmed using specific mimics and inhibitors for microRNA-21a and 126. Results showed that cell proliferation was inhibited by Pg (5mg/L-50mg gallic acid equiv. (GAE)/L) in colon cancer cells SW-480 and HT-29 and to a lesser extent in the non-cancer cells CCD-18Co (IC50 10, 16 and 26 mgGAE/L respectively), this was accompanied by a dose-dependent downregulation of inflammatory genes NF-kB and IL-6 mRNA and protein (down to 0.3 and 0.5 fold), and significant decrease in mRNA and protein level of cell adhesion molecule VCAM (0.4 down to untreated cells) was also observed. The downregulation of inflammatory genes was correlated with upregulation of miRNA-126a (up to 1.7 of untreated controls), that is involved in the post-transcriptional down-regulation of VCAM. Moreover, miRNA-21a which is involved in the regulation of cell cycle controlling factors was down regulated (down to 0.3 folds of untreated control) and the mimic increased the expression of microRNA-21 and downregulated targeted genes. Pg extract partially reversed the effects of the inhibitor and mimic. Overall, Pg had chemopreventive effects in colon cancer cells where microRNA-126a and microRNA21 seem to play a role in the underlying mechanism.

PS 2232 *IN VITRO* AND *IN VIVO* GENO – ANTIGENOTOXICITY EVALUATION OF WATERCRESS.

N. A. Casanova, M. M. Lopez Nigro and M. A. Carballo. *Bioquímica Clínica, Cigetox. Infibioc. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires, Buenos Aires, Argentina.* Sponsor: O. Olivero.

DNA damage seems to play a crucial role in several diseases and it is considered a probably carcinogenic factor. Consumption of vegetables might reduce oxidative stress. The objectives of this work were to develop an in vitro genotoxic study of watercress (*Rorippa Nasturtium-aquaticum*) through effect biomarkers: mitotic index, cellular proliferation kinetic, sister chromatid exchanges and comet assay; to evaluate its antigenotoxic properties against oxidative damage (in vitro comet assay) and to analyze genotoxicity and protective activity in vivo (comet assay and micronucleus test). The plant was chopped; the product was centrifuged, sterilized and

stored (-20 celsius degree). The concentrations assayed in vitro were calculated considering human intake (13.2 and 26.4 mg/ml; N=3). In the in vivo study, Swiss mice 7-8 weeks old (N=48) were treated with 0.5 and 1.0 g/kg body weight of watercress extract during 15 days by gavage. No statistically significant differences were detected for any of the biomarkers (p>0.05) indicating that the extract would not induce cytotoxicity, chromosomal instability, clastogenicity and DNA strand breaks. Significant antigenotoxic effects were observed for both concentrations (p=0.005 at 30 min; p<0.001 at 60 and 90 min), being the percentage of reduction of damage similar between them (67.1–75.2%). Moreover, the extract did not induce genotoxicity in vivo, exhibiting also a protective activity against cyclophosphamide induced damage (comet assay p<0.05; micronucleus test p<0.001). Our results constitute new knowledge about the diet impact on health; however, more studies are needed to explore the characteristics of watercress as a healthiness promoter.

PS 2233 CURCUMIN DECREASES INFLAMMATION IN NORMAL CELLS AND EXERT CHEMOPREVENTIVE EFFECT ON COLON CANCER CELLS THROUGH REGULATION OF KEY MICRORNAS.

G. D. Noratto[1, 2, 3] and S. U. Mertens-Talcott[1, 2, 3]. [1]*Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX,* [2]*Nutrition and Food Science, Texas A&M University, College Station, TX and* [3]*Institute for Obesity Research and Program Evaluation, Texas A&M University, College Station, TX.*

The anti-cancer and anti-inflammatory properties of curcumin have been investigated in multiple studies; however the effects of curcumin in regulation of microRNAs involved in the post-transcriptional regulation of pro-invasive and pro-inflammatory genes has not been much investigated yet. We studied the effects of a standardized curcuminoid extract in growth suppression and gene regulation of SW-480 cancer and non-cancer CCD-18Co colon cells. Cell proliferation and cell viability were assessed by cell counting and MTT assay respectively, microRNA and mRNA regulation by qRT-PCR, p-Glicoprotein activity by determination of Rh123 efflux2, protein expression by multiplex bead-based immunoassays and western blots. Results showed that curcumin at dose-range of 2.5-10 mg/L, preferentially suppressed growth, enhanced the drug absorption and decreased metalloproteinase 9 protein excretion on SW-480 cells, while decreased expression of inflammatory markers exerted by lipopolysacharide (LPS) in CCD-18Co. The cancer growth inhibitory effect of curcumin was mediated, at least in part by decreased expression of the anti-apoptotic survivin and the pro-invasive VEGF down to 0.3-fold, both are known to be regulated by specificity protein (Sp) transcription factors. In addition, Sp1, Sp3 and Sp4 were down-regulated by curcumin in a dose-dependent manner down to 0.6-, 0.7-, and 0.3-fold respectively. Moreover, repression of Sp and Sp-dependent genes by curcumin was correlated to the down-regulation of microRNA-27a (miR-27a) down to 0.3-fold and induction of zinc finger and BTB domain containing 10 (ZBTB10), an Sp repressor. Overall, these data confirm the anti-cancer effects of curcumin and its role as anti-invasive colon cancer, research on the use of miR-27 mimic and vectors is in progress to validate the role of curcumin as miR-27a-ZBTB10-Sp regulator.

PS 2234 13-WEEK GAVAGE EXPOSURE TO GUM GUGGUL EXTRACT (GGE) MODULATES ACTIVITY OF HEPATIC CYP2B AND CYP3A IN HARLAN SPRAGUE-DAWLEY RATS AND B6C3F1 MICE.

C. A. Granville[1], M. K. Vallant[2], M. Hejtmancik[1], D. K. Gerken[1] and M. E. Wyde[2]. [1]*Battelle Memorial Institute, Columbus, OH and* [2]*NTP/NIEHS, Research Triangle Park, NC.*

GGE is the oleoresin of the gum guggul tree, Commiphora mukul. GGE is currently used as a dietary supplement/remedy, and is marketed for the treatment of several indications, including tumors, hyperlipidemia, arthritis, obesity, coronary heart disease, and nodulocystic acne. Due to the potential for widespread and long term use and the limited toxicologic data available, the National Toxicology Program is assessing the toxicity of GGE in Harlan Sprague-Dawley rats and B6C3F1 mice. Groups of 20 rats/sex or 25 mice/sex were given 0, 15.5 (mice only), 31 (mice only), 62.5, 125, 250, 500 (rats only) or 1000 (rats only) mg GGE/kg body weight by gavage 5 days per week for up to 13 weeks with an interim termination after 4 weeks. For both species, no treatment-related mortality was observed, there were no abnormal clinical signs, and mean body weights were within 10 percent of control for animals in all dosage groups. GGE-related differences in organ and organ-to-body weight ratios were limited to the rat liver. There were no gross treatment-related observations noted for males or females in any dosage group for either species and microscopic evaluations are in progress. Activity of hepatic cytochrome P450 (CYP2B and CYP3A) was altered in both species. In mice, elevated CYP3A activity was observed in males and females treated for 4 or 13 weeks with 250 mg GGE/kg body weight, while elevated CYP2B activity was confined to males and females treated for 13 weeks with 250 mg GGE/kg body weight. In rats, dose-related increases in hepatic CYP3A activity was observed in males and females treated with all dosages of GGE after both 4 and 13 weeks of dosage. Dose-related increases in hepatic CYP2B activity were confined to males and females treated with 500 mg GGE/kg body weight and higher after both 4 and 13 weeks of dosage. The changes in CYP2B and CYP3A enzyme activity have clinical implications for alterations in the metabolism of other drugs and xenobiotics.

PS 2235 CURCUMIN REGULATES CELL CYCLE PROGRESSION IN RESPONSE TO BPDE-INDUCED DNA DAMAGE.

E. N. Rogers and J. States. *Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

Curcumin, a component of turmeric, decreases chemical carcinogen-induced DNA damage. It is suggested that activated p53, a tumor suppressor protein, inhibits carcinogenic activity by signaling cell cycle arrest, DNA repair, and apoptosis. Whether curcumin plays a role in p53-mediated cellular responses remains unclear. Therefore the aim of this study was to investigate whether curcumin prevents benzo[a]pyrene diol epoxide (BPDE)-induced damage by regulating p53-mediated cellular responses. Immunoslot blot assay showed that BPDE-DNA adducts were much lower in p53-expressing lung epithelial cells than in p53-deficient cells. In addition, BPDE-DNA adducts were lowered in p53-expressing cells pretreated with curcumin. Flow cytometry analysis revealed both cell lines accumulated in S-phase in response to BPDE-induced damage. While p53-expressing cells escaped S-phase arrest 12 h after BPDE treatment, p53-deficient cells entered and remained in S-phase. Curcumin pretreatment had little effect on the accumulation of p53-expressing cells in S-phase. On the other hand, curcumin pretreated p53-deficient cells entered S-phase arrest much earlier than cells treated with BPDE alone. Curcumin pretreated p53-expressing cells exhibited enhanced levels of p53 and p53 phosphorylated on serine 15 (p53S15) in a time-dependent manner in response to BPDE-induced damage. Phosphorylated retinoblastoma (P-pRb) was down-regulated with curcumin pretreatment. Interestingly, expression of CHK1 phosphorylated on serine 345, an upstream kinase targeting p53 as well as indicator of S-phase arrest, was much higher in curcumin pretreated p53-deficient cells than p53-expressing cells pretreated with curcumin. Expression of cyclin A, but not cyclin E, was reduced in a time-dependent manner in response to BPDE-induced damage only in p53-expressing cells. Collectively, these data suggest that curcumin may possess chemopreventive activity by regulating a variety of mechanisms including p53 activation as well as cell cycle progression in response to BPDE-induced damage. Supported by F31ES016719, R03CA119295 and P30ES014443.

PS 2236 ACUTE TOXICITY (LC50)AGAINST ARTEMIA FRANCISCANA OF ESSENTIAL OILS FROM *LANTANA ARMATA SCHAUER, XYLOPIA AROMATICA LAMARCK*, AND *SATUREJA BROWNEI BRIQ* COLLECTED IN COLOMBIA.

B. E. Jaramillo[1, 2], J. Olivero[1, 2], K. Caballero[2] and E. Stashenko[3]. [1]*Agrochemical Research Group, Chemical Program, Faculty of Sciences, University of Cartagena, Cartagena, Bolivar, Colombia,* [2]*Enviromental and Computational Chemistry Group (GQAC). Faculty of Pharmaceutical Sciences, University of Cartagena, Cartagena, Bolivar, Colombia and* [3]*National Center of Research for the Agro-industrialization of Aromatic Plant Species and Tropical Medicines-CENIVAM, Industrial University of Santander, Bucaramanga, Santander, Colombia.*

The medium lethal concentration (LC50) against Artemia franciscana is a simple, inexpensive, rapid and convenient method for assessment of relative biological activity. Essential oils from Lantana armata (Verbenaceae), Xylopia aromatica (Annonaceae) y Satureja Brownei (Lamiaceae) have been traditionally used for diverse purposes in different parts of the world. The most common use of these species is for the treatment of respiratory and gastrointestinal disorders, although some have shown analgesic,antipyretic, anti-inflammatory, antifungal, antibacterial and antihelminthic properties. In this work, essential oils from Lantana armata Schauer, Xylopia aromatica Lamarck y Satureja brownei Briq plants collected in Bucaramanga, Colombia have been screened for their lethal effects against Artemia franciscana, and selected oils have also been characterized by gas chromatography-mass spectrometry (GC-MS). Essential oil more toxic was L. armata, exhibited LC50 values of 3.8 and 2.1 μg/mL after 24 and 48 h exposures, respectively. The

essential oil of X. aromatica, presented an average toxicity (LC50 36.4-53.6 μg/mL). Essential oil less toxic against A. franciscana was S. brownie (LC50 = 76.1-111.4 μg/mL). The results of this screening, as well as the GC/MS characterization, indicates that these species are important sources of diverse natural products with potential pharmacological properties. the assayed essential oils could be inserted in further pre and clinical toxicological evaluation in order to establish a safe exploitation of their biological potentiality.

PS 2237 HEPATIC GENE EXPRESSION PROFILING IN PERFLUOROHEXANE SULFONATE-EXPOSED WILD-TYPE AND PPARα-NULL MICE.

M. B. Rosen[1], J. R. Schmid[2], K. P. Das[3], H. Ren[4], B. D. Abbott[3] and C. Lau[3]. *[1]Integrated Systems Biology, U.S. EPA, Research Triangle Park, NC, [2]Biostatistics and Bioinformatics, U.S. EPA, Research Triangle Park, NC, [3]Toxicology Assessment, U.S. EPA, Research Triangle Park, NC and [4]Genomics Research Core, U.S. EPA, Research Triangle Park, NC.*

Perfluorohexane sulfonate (PFHxS) is one member of a group of perfluoroakyl acids (PFAAs) presently recognized as widespread environmental contaminants. Like other PFAAs, PFHxS is also commonly found in human serum. Although PFHxS is presumed to be an activator of peroxisome proliferator-activated receptor alpha (PPARα), little is known about its toxicity. Male wild-type (WT) and PPARα-null (Null) mice were dosed by oral gavage with PFHxS (3 or 10 mg/kg/day), or vehicle for 7 days. Animals were euthanized, livers weighed, and liver samples collected for preparation of total RNA. Gene profiling was conducted on 4 mice per group using Affymetrix 430_2 broad expression microarrays and the results were contrasted to data previously collected by our laboratory for other PFAAs. In WT mice, exposure to PFHxS altered the expression of genes associated with a number of PPARα-regulated biological functions such as fatty acid metabolism, inflammation, peroxisome biogenesis, and proteasome biogenesis. Genes related to cholesterol biosynthesis were up-regulated in WT mice as well. In Null mice, changes induced by PFHxS reinforced findings from prior studies which indicated that in the absence of PPARα signaling, various PFAAs maintain regulation of genes related to fatty acid metabolism, including Cyp4a14 and certain peroxisomal genes. The constitutive androstane receptor (CAR)-regulated gene, Cyp2b10, was up-regulated in both WT and Null mice suggesting that, like other PFAAs, PFHxS is an inducer of CAR. These results demonstrate that the influence of PFHxS on the murine transcriptone does not deviate significantly from that observed for other PFAAs and that PFHxS has both PPARα-dependent and -independent activity. (This abstract does not necessarily reflect EPA policy.)

PS 2238 EFFECTS OF BIOREMEDIATION ON TOXICITY AND GENOTOXICITY OF PAH-CONTAMINATED SOIL USING GENETIC ENGINEERED CELL LINES.

J. Hu, J. Nakamura and M. D. Aitken. *Department of Environmental Sciences & Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC.*

Bioremediation is one of the commonly applied remediation strategies at sites contaminated with polycyclic aromatic hydrocarbons (PAHs). However, it remains controversial whether bioremediation could reduce health risk while removing the target compounds. This study investigated changes in the toxicity and genotoxicity of PAH-contaminated soil from a former manufactured-gas plant site before and after two simulated bioremediation processes: treatment in a laboratory sequencing batch reactor and long-term, continuous-flow columns (control and biostimulated columns). Soil samples included untreated soil for the bioreactor, untreated soil used to pack the columns, treated soil from the bioreactor, and treated soils from four different sample ports along the column length in each column. Genotoxicity of soil extracts was determined by multi-well plate-based DNA damage response analysis using the chicken DT40 B-lymphocyte isogenic cell line (DT40) and its DNA-repair deficient mutant (RAD54 knockout) cell line (RAD54). Bioreactor treated soil had lower LD50(DT40) than untreated bioreactor soil (0.82±0.10 and 1.29±0.05 mg dry soil/mL, respectively) while no significant difference was observed for LD50(RAD54) between these two soils (0.51±0.05 and 0.53±0.10 mg dry soil/mL, respectively). Treated soils from both the control and biostimulated columns had higher LD50(DT40) as well as LD50(RAD54) than untreated column soil (LD50(DT40): 4.32±0.16 and 1.94±0.16 mg dry soil/mL, respectively; LD50(RAD54): 1.78±0.12 and 1.25±0.01 mg dry soil/mL, respectively). Biostimulated column soils had both higher LD50(DT40) and LD50(RAD54) than control column soils at corresponding sample ports, but there was no significant difference in LD50 RAD54/DT40 ratio among these soils. These results suggest that bioremediation does not necessarily reduce the toxicity or genotoxicity of PAH-contaminated soil, and that different bioremediation processes can have different effects on residual toxicity or genotoxicity.

PS 2239 PERFLUOROPHOSPHONIC ACID ACTIVATES PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA BUT NOT CONSTITUTIVE ANDROSTANE RECEPTOR IN THE MURINE LIVER.

K. P. Das[1], M. B. Rosen[2], C. R. Wood[1], B. Abbott[1] and C. Lau[1]. *[1]Toxicity Assessment Division, ORD, NHEERL, U.S. EPA, Research Triangle Park, NC and [2]Integrated System Toxicity Division/NHEERL/ORD, U.S. EPA, Research Triangle Park, NC.*

Masurf FS-780 is a commercial perfluoro-chemical mixture that contains C6-12-perfluoroalkylphosphonic acid (PFPA) derivatives. PFPAs have received recent attention as a previously under recognized subclass of perfluoroalkyl acids (PFAAs) that are found in the environment. The current study was designed to evaluate liver toxicity of PFPAs and to contrast these findings to studies previously conducted by our group for other PFAAs. Two independent experiments were conducted. In experiment one, adult male CD-1 mice were given Masurf FS-780 once daily for 7 days by oral gavage at 10.4, or 41.6 mg/kg. In a second experiment, SV129 wild-type (WT) and peroxisome proliferator-activated receptor alpha (PPARα)-null male mice (Null) were similarly dosed with Masurf FS-780 at 3.1 or 20.8 mg/kg/day. Mice from each dose group, along with concurrent controls, were sacrificed 24 h after the last treatment. Liver samples were collected for real time RT-PCR of selected genes using Taqman assays. Dose-dependent elevations of liver weight were found in CD-1 and SV129 WT mice but not in Null mice. Similarly, genes known to be regulated by PPARα were up-regulated in a dose-dependent fashion in CD-1 and SV129 WT mice but, with the exception of Cyp4a14, were unchanged in Null mice. Hence, like other PFAAs, PFPAs appear to function as an activators of PPARα. Unlike other PFAAs (perfluorooctanoic acid, perfluorooctane sulfonate, perfluorononanoic acid), Masurf FS-780 does not up-regulate the constitutive androstane receptor (CAR) regulated gene, Cyp2b10, in either strain of mice examined suggesting that PFPAs may not activate CAR. These results suggest that PFPA-induced liver enlargement occurs specifically through activation of PPARα. (This abstract does not necessarily reflect US EPA policy.)

PS 2240 ACTIVATION OF MOUSE AND HUMAN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA (PPARα) BY PERFLUOROALKYL ACIDS (PFAAS) OF 5, 7, 8, 11, AND 12 CARBON CHAIN LENGTHS IN COS-1 CELLS.

C. J. Wolf, C. Lau and B. D. Abbott. *Toxicity Assessment Division, U.S. EPA, ORD, NHEERL, Research Triangle Park, NC.*

PFAAs are surfactants that have been found globally in the environment and in tissues of humans and wildlife. They adversely affect perinatal survival and development in rodents and PPARα is involved in inducing these effects. Our previous study demonstrated that some PFAAs activate PPARα in transiently transfected COS-1 cells. Here we test additional PFAAs for their ability to activate mouse and human PPARα. COS-1 cells were transfected with either a mouse or human PPARα-responsive luciferase reporter plasmid. After 24 hours, cells were exposed to either vehicle control (0.1 % DMSO or water), PPARα agonist (WY14643, 10 μM), perfluoropentanoic acid (C5), perfluoroheptanoic acid (C7), perflouroctanoic acid (C8), perfluoroundecanoic acid (C11), or perfluorododecanoic acid (C12) at thirteen concentrations from 0.5 - 100 μM. After 24 hours of exposure, cells were lysed and luciferase activity was measured. Data were analyzed using ANOVA and linear regression analysis. Relative PFAA activities were compared using C20max, the concentration at which each PFAA produces 20% of the highest response elicited by the most active PFAA for each species. C8 induced the highest activity in the human PPARα, followed by C7, C5, and C11 (C20max, 4.5 μM, 13 μM, 45 μM and 126 μM). C12 had little activity (C20max could not be calculated). However, the highest activity in the mouse PPARα was induced by C12 followed by C8, C11, C7 and C5 (C20max, 5.4 μM, 7.2 μM, 9.2 μM, 11 μM, and 35 μM, respectively). While each PFAA activated the mouse and human PPARα, they generally induced less activity in human than in mouse PPARα. We found a pattern of increasing activity with increasing chain length of the PFAA up to C8 and low activity with longer chain PFAAs (C11 and C12) with human PPARα, a pattern we reported previously. This study completes our survey of the ability of PFAAs from C4 through C12 to induce PPARα activity in vitro. This abstract does not necessarily reflect EPA policy.

PS 2241 DDA, A BIOMARKER FOR ENVIRONMENTAL DDT EXPOSURE.

Z. Chen[1], H. H. Vega[1], S. M. Patrick[2], M. S. Bornman[2] and R. I. Krieger[1]. *[1]Personal Chemical Exposure Program, Department of Entomology, Environmental Toxicology Graduate Program, University of California-Riverside, Riverside, CA and [2]Department of Urology, University of Pretoria, Steve Biko Academic Hospital, South Africa.*

DDT is the best known POP. It is more commonly measured as "DDTs (an ill-defined mixture of DDE, DDD, and DDT)". The analytical convenience of this designation contrasts with the toxicology of DDT and its terminal metabolite, DDE. Since DDT is converted to DDA and excreted in urine of humans and feces of birds, the biomarker is a means to recognize current DDT exposure for environmental and forensic applications. DDT feeding studies (10-3000 ppm DDT) produced DDA in chicken feces at levels of 0.02-19.43 ug DDA/g during and after DDT feeding periods. Chicken eggs were collected daily and analyzed for DDT related compounds. DDA was not found in eggs. Hexane extracts of egg yolk contained DDT (5-6785 ng/g) and DDE (1-4044 ng/g) by GC-ECD analysis. DDD levels were low (<600 ng/g). DDA levels in feces positively correlated with egg DDT levels. The Pearson's correlation coefficient (r) is 0.9915. The p-value is highly significant ($p<0.001$). DDT is used in indoor residual spraying program in South Africa for malaria control. Chickens may have environmental DDT exposures. Whole eggs from chickens in a sprayed area contained DDT (2611-17320 ng/g) and DDE (2599-30619 ng/g). Highest DDD levels were 17 ng/g. "DDTs" were below 107 ng/g in the control area (Unpublished, personal communication). Similar DDT levels in eggs from Africa and our feeding trials indicate current DDT exposure. We expect DDA in feces of chickens in Africa due to the occurrence of DDT in eggs. In the Los Angeles Bight, a region of heavy DDT contamination, brown pelican feces contained only DDE. Determination of DDA in environmental bird feces can be used to distinguish DDT and DDE exposure. Since DDT is strictly regulated wherever used, its occurrence easily becomes an environmental issue. Demonstration of DDT exposure using DDA as a biomarker represents a useful tool to clarify some public health and regulatory concerns.

PS 2242 STRUCTURE-ACTIVITY RELATIONSHIP FOR INDUCTION OF CYTOCHROMES P450 BY NON-DIOXINLIKE POLYCHLORINATED BIPHENYLS IN CULTURED RAT HEPATOCYTES.

M. Gährs, R. Roos and D. Schrenk. *Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany.*

There is no clear structure-activity relationship for the various toxic effects of non-dioxinlike polychlorinated biphenyls (NDL-PCBs), a relevant subgroup of persistent environmental pollutants. Many NDL-PCBs are 'phenobarbital-type' inducers of hepatic cytochrome P450 (CYP) enzymes, mainly of the CYP2 and 3 families, in humans and experimental animals. Induction of these enzymes is mediated by the nuclear xenobiotic receptors CAR (constitutive active receptor) and PXR (pregnane-X-receptor). The former is also thought to play a central role in the liver tumour-promoting effects of 'phenobarbital-type' inducers of drug metabolism. In primary rat hepatocytes, incubation with 0.1 mM phenobarbital over 24 h in Williams' E medium supplemented with insulin and dexamethasone resulted in a several hundred fold induction of CYP2B1 mRNA and an about six-fold induction of CYP3A1 mRNA, whereas culture in DMEM prevented induction completely. Furthermore, a strong induction of both mRNAs was obtained with the six most abundant NDL-PCBs, congeners 28, 52, 101, 138, 153, and 180. Induction by PCBs but not by phenobarbital was further increased when hepatocytes isolated under collagenase-free conditions were used. Concentration-response analysis revealed a very narrow window of EC50 estimates for CYP2B1 induction, being in the range of 1-10 μM for PCBs 28, 52, 101, and 138, and between 0.1 and 1 μM for PCBs 153 and 180. CYP3A1 induction was less sensitive to NDL-PCBs, the most pronounced induction being achieved at 100 μM with the higher chlorinated congeners. Our findings suggest that induction of CYP2B1 and CYP3A1 mRNAs by NDL-PCBs, a hallmark of CAR/PXR-mediated hepatic effects, is not highly structure-dependent. Higher chlorinated congeners such as PCBs 153 and 180 seem to be about tenfold more potent in inducing CYP2B1 and about twice as effective in inducing CYP3A1 than the lower chlorinated congeners.

PS 2243 STRUCTURAL AND FUNCTIONAL INJURY INDUCED BY FLUORINATED ORGANIC COMPOUNDS IN PREFRONTAL CORTEX OF MALE RAT.

A. Lafuente[1], A. Caride[1], N. Pereiro[1], V. Franco[1], R. Moyano[2] and A. Anadón[3]. *[1]Laboratory of Toxicology, Faculty of Sciences, Universidad de Vigo, Orense, Spain, [2]Department of Pharmacology, Toxicology, Legal Medicine and Forensic Sciences, Universidad de Córdoba, Cordoba, Spain and [3]Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain.*

Perfluorooctane sulfonate (PFOS), a persistent and bioaccumulative compound, is widely distributed in the environment. It has been shown to be neurotoxic in experimental animals. This study was designed to explore the possible PFOS-induced alterations of biogenic amine concentration in prefrontal cortex. Dopamine and serotonin metabolisms were also studied. Morphological changes were also evaluated. For this purpose, male Sprague-Dawley rats were orally treated for 28 days with PFOS, at the doses of 0.5, 1, 3 and 6 mg/kg/day. Control rats received 2.0% Tween-20 vehicle. Biogenic amines were measured by HPLC with electrochemical detection, and histological study was performed by using light and electron microscopy. PFOS, at the dose of 1.0 and 3.0 mg/kg/day, increased epinephrine concentration. Dopamine content was higher in rats treated with 1.0 mg PFOS/kg/day than in control animals, and its intraneural metabolism was increased after exposure to the dose of 3 mg/kg/day. PFOS modified serotonin concentration and its metabolism. More concretely, the dose of 1.0 mg/kg/day increased serotonin concentration and decreased its metabolism, and exposure to 0.5 mg/kg/day also decreased serotonin metabolism. The histopathological study highlights degenerative processes, mainly at the molecular layer; very basophilic neurons, vacuolar degeneration and satellitosis. These results suggest that fluorinated organic compounds induce several modifications on biogenic amine concentration and metabolism, as well as morphological alterations, at prefrontal cortex level.

PS 2244 GENDER SPECIFIC DIFFERENCES IN LEVELS OF PERSISTENT ORGANIC POLLUTANTS IN AN ELDERLY POPULATION IN SWEDEN – PROSPECTIVE INVESTIGATION OF THE VASCULATURE IN UPPSALA SENIORS (PIVUS).

S. Salihovic[1], L. Mattioli[1], G. Lindström[1], P. Lind[2], L. Lind[3] and B. van Bavel[1]. *[1]School of Science and Technology, Örebro University, MTM Research Centre, Örebro, Sweden, [2]Department of Medical Sciences, University Hospital, Occupational and Environmental Medicine, Uppsala, Sweden and [3]Department of Medical Sciences, University Hospital, Acute and Internal Medicine, Uppsala, Sweden.*

INTRODUCTION: Persistent organic pollutants (POPs) are diverse synthetic chemicals that are resistant to degradation and accumulate throughout the food chain. Therefore, they are widely spread in the environment and can be found in remote areas such as the arctic. The aim of the study was as follows: (1) to investigate concentrations of 21 different POPs in human plasma samples from seniors residing in the city of Uppsala, Sweden, (2) to evaluate gender differences in relation to the levels of the POPs and extensive health data acquired from the subjects. Screening was focused on those pollutants listed in the Stockholm Convention (2001). MATERIALS AND METHODS: Blood plasma samples were collected from 1016 (50.2% female) individuals, all aged 70 years old, living in the city of Uppsala, Sweden. Solid-phase extraction (SPE) followed by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC/HRMS) was used to analyze 21 target compounds including polychlorinated biphenyls (PCBs), octachlorodibenzodioxin (OCDD), chlordanes, hexachlorobenzene (HCB), dichlorodiphenyldichloroethylene (DDE) and polybrominated diphenylether (PBDE). RESULTS: Wide ranges of POPs were detected in the samples; both PBDE and DDE were found in concentrations similar to what has been observed in other studies in Europe. Gender specific differences in POP levels were statistically confirmed, suggesting that that levels of PCBs, trans- nonachlordane, OCDD and BDE-47 differ between men and women. CONCLUSION: Differences in POP levels between men and women in the cohort were seen in the lipid normalized data. Surprisingly the levels in female participants were not always lower as could be expected after breast feeding earlier in life.

PS 2245 POLYCHLORINATED BIPHENYL 153 WORSENS DIET-INDUCED OBESITY AND NON-ALCOHOLIC FATTY LIVER DISEASE IN C57/BL6 MICE.

M. Cave[1, 2], B. Gregory[1], R. McCAllister[1], K. Falkner[1], D. Conklin[1], D. Young[1], C. McClain[1, 2] and A. Bhatnagar[1]. *[1]Department of Medicine/GI, University of Louisville, Louisville, KY and [2]Robley Rex Veterans Administration Medical Center, Louisville, KY.*

Introduction: Polychlorinated Biphenyls (PCBs) are persistent environmental pollutants. We recently reported that multiple PCBs, including PCB 153, were dose-dependently associated with increased odds ratios for serum alanine aminotransferase elevation, a biomarker for suspected nonalcoholic fatty liver disease (NAFLD), in adult participants in the National Health and Nutrition Examination Study. PCB 153 had the highest median serum concentration of any single PCB. The purpose of this study is to determine if PCB 153 induces NAFLD in mice fed a normal chow diet, and worsens NAFLD in mice fed a high fat diet. Methods: C57/BL6 mice were fed either a control or 43% milk fat diet for 12 weeks with or without PCB 153 co-exposure (50 mg/kg i.p. x 4). A glucose tolerance test was performed, and serum and tissue were obtained at necropsy for measurement of adipocytokine levels and histology. Results: PCB 153 treatment was associated with the development of microvesicular hepatic steatosis in animals fed the control diet, and it worsened macrovesicular steatosis in animals fed the high fat diet. PCB 153 treatment worsened the diet-induced obesity observed in mice fed the high fat diet, and this appeared to be mostly due to increased central and visceral adiposity. PCB 153 did not affect insulin resistance or glucose intolerance on either diet. Adipokines were increased by high fat feeding, and these effects were augmented by PCB 153 co-treatment. Conclusions: PCB 153 acts synergistically with high fat feeding to promote central adiposity and NAFLD. These data suggest that the epidemiologic association between PCBs and NAFLD may be causal. Nutrient-PCB interactions may be critical determinants of PCB-related disease. Funding Information: NIEHS (P30ES014443-01A1, T35ES014559), the NIAAA (K23AA18399-01A, 1P01AA017103-01), and NCRR (5P20RR024489-02).

PS 2246 TETRABROMOBISPHENOL A, BUT NOT BISPHENOL A, IS A POTENT MODULATOR OF THE TYPE 1 RYANODINE RECEPTOR.

R. Zhang and I. N. Pessah. *Department of Molecular Biosciences, School of Veterinary Medicine, UC Davis, Davis, CA.*

Tetrabromobisphenol A (TBBPA) is a widely used brominated flame retardant (BFR) utilized primarily in electronic equipment. The global annual demand for TBBPA is more than 120,000 metric tons, accounting for half of all BFRs produced. TBBPA is a brominated derivative of bisphenol A (BPA), a compound capable of disrupting endocrine signaling. Although TBBPA may also exhibit weak estrogenic effects, Reistad and coworkers recently demonstrated that TBBPA disrupts Ca2+ homeostasis in cerebellar granule neurons. Here we report that TBBPA is a potent modulator of the type 1 ryanodine receptor (RyR1). The effects of TBBPA on RyR1 was investigated using [3H]ryanodine binding analysis and microsomal Ca2+ flux measurements. In the binding assay, TBBPA elicited an increase of specific [3H]ryanodine binding to RyR1 at 37°C, with a threshold concentration of 3nM (~140% of vehicle control, p=0.028) and reaching ~500% of vehicle control at 30μM, suggesting potent activation of RyR1 channels. Functional measurement with actively loaded microsomal vesicles in the presence of Mg-ATP indeed showed TBBPA-induced rapid efflux of accumulated Ca2+. Ca2+ release was observed upon addition of TBBPA as low as 1μM, with 30μM TBBPA inducing initial release rates of approximately 1.5nmol Ca2+/s. This is in contrast to BPA (<30μM), which elicited negligible release of Ca2+ actively accumulated by microsomes. At 30μM, the initial release rate of Ca2+ elicited by TBBPA was ~70-fold that observed with BPA. The total amount of intravesicular Ca2+ released after 5 minutes was ~30-fold greater for TBBPA compared to BPA. TBBPA-induced Ca2+ release was fully blocked with brief pre-incubation of the microsomes with ruthenium red, a RyR1 blocker. These effects of TBBPA on RyR1 are similar to those previously reported for non-coplanar polychlorinated biphenyls and brominated diphenylethers, suggesting a convergent, possibly additive mechanism. Sponsored by NIH P42 ES04699, P01 11269, and U.S. EPA R833292 and R829388.

PS 2247 HUMAN ORGAN ANION TRANSPORTING POLYPEPTIDES (OATPS) 1B1, 1B3 AND 2B1 TRANSPORT PERFLUOROCARBOXYLIC ACIDS.

B. Hagenbuch[1], M. Boissier[1], Y. M. Weaver[1], D. J. Ehresman[2], S. Chang[2] and J. L. Butenhoff[2]. *[1]Pharmacology, The University of Kansas Medical Center, Kansas City, KS and [2]Medical Department, 3M Center, St. Paul, MN.*

Perfluorocarboxylates (PFCAs) are stable surfactants and fatty-acid analogues many of which have been detected broadly in environmental matrices, including serum and liver of humans and wildlife. In rats, the shorter perfluoroheptanoate (C7) and perfluorooctanoate (C8) are excreted via the kidneys while the longer perfluorononanoate (C9), perfluorodecanoate (C10) and perfluoroundecanoate (C11) accumulate in the liver. The rat and human transporters involved in renal excretion have recently been identified and characterized and mainly belong to the *SLC22A/Slc22a* family of organic anion transporters. However, it is not known whether similar transporters are also involved in liver accumulation. Therefore, we wanted to test which of the human liver expressed organic anion transporting polypeptides (OATPs) that belong to the *SLCO* family, could be involved in the uptake of PFCAs into hepatocytes. We used cell lines stably expressing human OATP1B1, OATP1B3 and OATP2B1, and measured uptake of the model substrates estrone-3-sulfate or estradiol-17β-glucuronide in the absence and presence of PFCAs with chain lengths from two to eighteen carbons. PFCAs with a carbon chain length of 9 to 11 were the most potent inhibitors. Direct uptake measurements of these PFCAs revealed that OATP2B1 mainly transported C8. The liver specific OATP1B1 mainly transported C9 while OATP1B3 transported C8 to C11 but minimally transported C7. Additional preliminary kinetic studies with OATP1B1 and OATP1B3 suggested that the affinity for PFCAs decreased while the maximal transport rate increased with increasing chain length. In conclusion, these results suggest that liver accumulation of the longer chain PFCAs could be due to the fact that these compounds are substrates of OATPs.

PS 2248 METABOLOMIC ANALYSIS OF SERUM AFTER TREATMENT WITH THE EMERGING POP FLAME RETARDANT HEXABROMOCYCLODODECANE (HBCD): COMMERCIAL MIXTURE, ALPHA AND GAMMA STEREOISOMERS ELICIT DIFFERENTIAL EFFECTS IN INFANTILE MICE.

D. T. Szabo[1, 2], W. Pathmasiri[3], J. J. Diliberto[2], S. Sumner[3] and L. S. Birnbaum[4]. *[1]University of North Carolina, Chapel Hill, NC, [2]U.S. EPA, Research Triangle Park, NC, [3]RTI International, Research Triangle Park, NC and [4]NCI/NIEHS, Research Triangle Park, NC .*

HBCD is an emerging persistent organic pollutant flame retardant added to foam used in building insulation, electronics, and textiles. The commercial mixture is composed of 3 stereoisomers: alpha (10%); beta (10%); gamma (80%). A shift from the dominant gamma in the commercial mixture to alpha in humans and biota is observed. To aid in predicting health risks of HBCD, metabolomic analysis of serum was performed to improve our ability to draw correlations between early life exposures and developmental outcomes. Metabolomics examines low molecular weight endogenous compounds, and can give a snapshot of the physiological profile. Ten day old female C57BL/6 mice were exposed to a single gavage dose of corn oil vehicle, 3, or 30 mg/kg of the commercial HBCD mixture, alpha or gamma HBCD. These concentrations of HBCDs induce effects at the molecular level in infant pups. Serum was collected 4 days postexposure on postnatal day 14 (pnd 14). Samples were prepared for metabolomic analysis by mixing 60 μL of pnd 14 trunk serum with saline, D2O, and formate as an internal control. 1H NMR spectra were acquired using a 950 MHz NMR. Multivariate analysis was conducted for NMR data processed by the traditional binning approach and via a concentration library matching approach. Four days after oral exposure to a single dose, metabolic profiles in serum could differentiate pups that received the vehicle, HBCD commercial mixture, alpha or gamma. Metabolites significant to the separation of dose groups were mapped to biochemical pathways finding significant metabolic perturbations in amino acid and energy metabolism. Metabolomics is a promising approach to determine biomarkers for examining the mechanistic link between low levels of environmental exposure and disease/dysfunction. Abstract does not reflect EPA or NCI/NIEHS policy.

PS 2249 INTERACTION BETWEEN DIETARY SELENIUM LEVELS AND PCB126 EXPOSURE ON PON1 GENE EXPRESSION AND ACTIVITY IN RATS.

H. Shen[1], B. Wang[1], I. Lai[1], L. W. Robertson[1, 2] and G. Ludewig[1, 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.*

Cardiovascular diseases are a major cause of morbidity and mortality. The antioxidant benefits of selenium (Se), an essential trace element, on cardiovascular diseases have been appreciated for decades. Paraoxonase 1 (PON1), a known antioxidant enzyme, was also reported to have anti-atherogenic effects. Dioxin-like PCBs on the other hand were shown to cause oxidative stress and cardiovascular toxicity. We previously reported that PCB126 (3, 3', 4, 4', 5 – pentachloro- biphenyl), the most potent dioxin-like PCB congener, decreased hepatic Se levels. We also observed an effect of PCBs on hepatic and serum PON1 levels. In this study we examined the influence of dietary Se on PON1 levels and activities following PCB126 treatment. Male Sprague-Dawley rats, fed diets with differing Se levels (0.02, 0.2, or 2ppm), were treated with either a single intraperitoneal injection of corn oil or PCB126 (0.2, 1 or 5μmol/kg body weight) and euthanized two weeks later. At all 3 Se levels hepatic PON1 mRNA, protein level and activity as well as serum PON1 activities were significantly increased by PCB126 treatment ($P<0.01$) as were the hepatic mRNA levels of AhR, CYP1A1, and ApoA1 ($P<0.01$). No significant change was found in liver and serum TBARS levels. A significant inverse relationship was observed between dietary Se levels and hepatic PON1 mRNA levels, PON1 activities, and TBARS levels. Further statistical analysis (General Linear Models in SAS 9.2) revealed the strong interactions between Se and PCB126 for most of the hepatic endpoints investigated ($p < 0.01$). Our results indicate PON1 as a new potential biomarker of exposure. Furthermore, dietary Se levels significantly affected oxidative stress, PON1 activity changes, and gene induction caused by PCB126-treatment. The potential of PON1 to protect against xenobiotics and oxidative stress warrants further investigation (Supported by NIEHS P42 ES013661 and DOD DAMD17-02-1-0241)

PS 2250 PCB77 DECHLORINATION MIXTURES MODULATE PROINFLAMMATORY EVENTS IN VASCULAR ENDOTHELIAL CELLS.

K. Eske, C. Barton, A. Palumbo, S. Han, Z. Majkova, E. Zahran, L. Bachas, D. Bhattacharyya, M. Toborek and B. Hennig. *Superfund Research Program, University of Kentucky, Lexington, KY.*

Polychlorinated biphenyls (PCBs) are persistent organic pollutants consisting of a biphenyl with chlorine substititutions. Because of detrimental health outcomes, associated with exposure to PCBs, they represent a high priority for remediation at Superfund sites throughout the United States. We hypothesize that PCB dechlorination to biphenyl removes biological activity and toxicity in vascular endothelial cells. In the natural environment, complete dechlorination is difficult to achieve, and mixtures of PCBs result during remediation. Therefore, a study of three mixtures of PCB77 breakdown products was conducted to determine how the resulting biological activities differ from the parent PCB. Using data from time points within the dechlorination process, mixtures representing remediation products at 0 (PCB77), 10, 24, and 48 hours (biphenyl) were produced. These mixtures, as well as individual PCBs produced as a result of the dechlorination process, were tested for inflammatory endpoints. The remediation mixtures demonstrated a significant increase in CYP1A1 protein and mRNA expression compared to vehicle and biphenyl. In contrast, mRNA expression of VCAM-1 and MCP-1 followed a cyclical pattern, suggesting that remediation mixtures also have proinflammationry profiles. Except for the biphenyl, all dechlorination mixtures contributed to increased ROS production in endothelial cells. Our data also suggest that proinflammatory patterns of dechlorination mixtures may be caused mostly by PCB15, which is produced at the highest level after 10 and 24 hours of dechlorination. These data suggest that biological activities of remediation products should be tested to determine which degree of remediation sufficiently attenuates toxicity. (Supported in part by ARRA funds and P42ES007380).

PS 2251 TELOMERE DYSFUNCTION AND TELOMERASE REACTIVATION IN HUMAN SKIN KERATINOCYTES: A POSSIBLE NEW MECHANISM OF PCB CARCINOGENESIS.

P. Senthilkumar, A. Klingelhutz, L. Robertson and G. Ludewig. *Human Toxicology, The University of Iowa, Iowa City, IA.*

Activation of telomerase activity and lengthening of telomeres are key steps in the process of malignant cell transformation. Polychlorinated Biphenyls (PCBs), a group of 209 different congeners, are classified as probable human carcinogens. To explore if PCBs affect telomerase and telomeres, immortal human skin keratinocytes (HaCat) were exposed to PCB congeners 28, 52, 126 and 153 at 5μM concentration for 48 days. Medium with compound was changed every 3rd day and every 6th day cells were re-seeded at low density and telomerase activity and telomere length by qPCR, cMYC, hTERT, hTR and CYP1A1 gene expression by RT-PCR, CYP1A1 activity by EROD production, cell cycle distribution by flow cytometry, and superoxide level by DHE oxidation were determined. All PCB congeners reduced telomerase activity and telomere length. PCB126 caused the most prominent reduction of telomerase activity (50%), hTR and hTERT gene expression (10%), telomere length (40%) and cell growth, along with an increased number of cells in S-phase, an increase in CYP1A1 mRNA and activity, and in superoxide levels from day 6 to 48; cMYC levels were not affected. Treatment with PCB126 was continued and from day 54 on a change in growth behavior was observed. An astounding finding that an increase in cMYC, hTERT, and hTR gene expression level (to 130%) along with re-activation of telomerase activity (to 100%) and re-elongation of telomere length (to 90%) from day 54 to 90 was observed. This increase in cMYC, hTERT, and hTR transcripts after critical telomere shortening may be an indication of genomic instability, a hallmark of carcinogenesis. This study shows for the first time that PCBs initially reduce telomerase activity, telomere length, and cell growth, with possible mechanistic connections to increased CYP1A1 and oxidative stress, but can later lead to telomerase re-activation, telomere lengthening and increased cell growth, all key components in cancer initiation and progression (Supported by NIEHS P42 ES013661).

PS 2252 GLOBAL ASSESSMENT OF POLYBROMINATED DIPHENYL ETHERS IN SPERM WHALE (*PHYSETER MACROCEPHALUS*) SKIN.

L. Savery[1, 2, 3], S. Eagle[4], H. M. Stapleton[4], I. Kerr[5], W. Thompson[2, 3] and J. P. Wise, Sr.[1, 2, 3]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME, [2]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME, [3]Department of Applied Medical Sciences, University of Southern Maine, Portland, ME, [4]Nicholas School of the Environment, Duke University, Durham, NC and [5]Ocean Alliance, Lincoln, MA.*

Polybrominated diphenyl ethers (PBDEs) are industrial chemicals that persist and bioaccumulate and are now ubiquitous in the environment. They accumulate in fatty tissue and have the propensity to disrupt thyroid hormones and cause neurodevelopmental effects. Little data are available about the impact and extent of PBDEs in the ocean ecosystem. The sperm whale (*Physeter macrocephalus*), an endangered species, is a sentinel of ocean health. The aim of the study was to measure the levels of 25 PBDEs and two methoxylated PBDEs, likely of a natural origin, in sperm whale skin samples across the globe. Samples were collected from 100 free-ranging, adult, female sperm whales in seven regions during the voyage of the research vessel, The Odyssey, between 2000 and 2005. PBDEs were detected in 98% of the samples with concentrations ranging from less than MDL to 89.1 ng/g. BDE 47 was the dominant congener detected in most samples, while BDE congeners 28, 66, 99, 100 and 154 also contributed to the total PBDE content. Mean BDE 47 concentrations were highest in samples collected from the Mediterranean (13.3 ng/g ww, n=7) whereas samples collected from the Pacific had the lowest concentrations (2.8 ng/g ww, n=8). The regions with the highest total mean levels of PBDEs were the Mediterranean, Atlantic and Seychelles with 36.9, 12.6 and 12.5 ng/g ww, respectively. The two Me-O-PBDEs (6-MeO PBDE 47 and 2-MeO PBDE 68) had levels orders of magnitude higher than the man-made PBDEs with the highest levels in Papua New Guinea with 137.2 and 78.8 ng/g ww, respectively, and in Seychelles 100.3 and 63.3 ng/g ww, respectively. These data help to establish a global baseline for PBDE exposure in sperm whales and marine mammals, in general.

PS 2253 UNDERSTANDING THE TOXICITY OF BROMKAL 70-5DE, A PBDE FLAME RETARDANT MIXTURE, USING A YEAST MODEL.

B. Gaytan, C. Monroy, A. Loguinov and C. Vulpe. *University of California at Berkeley, Berkeley, CA.*

Polybrominated diphenyl ethers (PBDEs) are a group of synthetic organic brominated compounds that have been utilized to fire retard consumer products such as furniture, textiles, and electronics. PBDEs are persistent environmental contaminants increasingly detected across the globe in biota, air, and sediment, as well as human tissue, milk, and blood. Concerns have been raised over the toxicological risk associated with exposure, as various studies have linked PBDEs to developmental neurotoxicity, immunotoxicity, endocrine disruption, and cancer. In this study, it was hypothesized that the *Saccharomyces cerevisiae* gene deletion library

could help elucidate the mechanisms of toxicity associated with exposure to Bromkal 70-5DE, a technical pentabrominated diphenyl ether (pentaBDE) flame retardant mixture. Preliminary analyses have identified *HNT3Δ* as sensitive to the Bromkal 70-5DE pentaBDE mixture. *HNT3* is a DNA 5' AMP hydrolase involved in DNA repair and a homolog of human aprataxin, a Hint related protein mutated in individuals with ataxia-ocular apraxia. Future investigations will further examine the mechanisms by which deletion of *HNT3* affects the toxicity of Bromkal 70-5DE in yeast.

PS 2254 THE GENDER DIFFERENCE IN SENSITIVITY TO ACUTE TOXICITY OF TCDD IS REVERSED IN MICE COMPARED WITH RATS.

R. Pohjanvirta[1], H. Miettinen[2], S. Sankari[3] and J. Lindén[1, 4]. [1]*Department of Food Hygiene and Environmental Health, University of Helsinki, University of Helsinki, Finland,* [2]*Department of Environmental Health, National Institute for Health and Welfare, Kuopio, Finland,* [3]*Central Laboratory of the Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland and* [4]*Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland.* Sponsor: M. Viluksela.

Acute toxicity of the ubiquitous environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is known to vary widely among species and strains. Previous studies in rats have established that females are about 2-fold more sensitive to TCDD lethality than males. However, surprisingly little is known about possible gender-related sensitivity differences in mice. Using three substrains of TCDD-sensitive C57BL/6 mice and transgenic mice on the same background we found that: 1) In contrast to rats, in mice females represent the more resistant gender; 2) the magnitude of the divergence between male and female mice depends on substrain but can amount to over 10-fold; 3) mutations in the primary structure of the AH receptor (AHR) are not involved in the resistance of female mice of a C57BL/6 substrain despite that their acute LD50 for TCDD is over 5,000 μg/kg; 4) transgenic mice that express globally the rat wildtype AHR follow the mouse-type of the gender difference; 5) the severity of the lesion in liver (as evidenced by plasma ALAT values) developed during the first six days after TCDD exposure correlates best with the TCDD sensitivity of the genders; and 6) in mice, ovarian estrogens appear to confer TCDD resistance whereas testicular androgens enhance susceptibility to TCDD lethality. Hence, the two closely related rodent species most often employed in toxicological risk characterization studies, rat and mouse, represent opposite examples of the influence of gender on dioxin sensitivity further complicating the risk assessment of halogenated aromatic hydrocarbons.

PS 2255 REVERSE CAUSATION OF DIOXIN DOSE-RESPONSE TRENDS FOR RISK OF DIABETES MELLITUS TYPE 2 AMONG OPERATION RANCH HAND VIETNAM VETERANS.

B. D. Kerger[1], P. K. Scott[2], M. Pavuk[3], M. Gough[4] and D. J. Paustenbach[5]. [1]*Health Science Resource Integration, Tallahassee, FL,* [2]*ChemRisk, Pittsburgh, PA,* [3]*Centers for Disease Control and Prevention, Atlanta, GA,* [4]*Private Consultant, Berkeley, CA and* [5]*ChemRisk, San Francisco, CA.*

Apparent dose-response relationships between serum 2,3,7,8-tetrachlorodibenzo-p-dioxin levels (dioxin) and increased occurrence and severity of adult diabetes mellitus type 2 (DM) have been reported among U.S. Vietnam veterans in the Operation Ranch Hand (RH) cohort. This study examines the hypothesis that DM onset may lead to higher serum dioxin (reverse causation) rather than higher serum dioxin leading to DM (causation). RH diabetics with 2 or more serum dioxin measurements are evaluated for kinetic trends with DM onset and for frequency of DM risk factors including age, race, DM family history, and body mass index. Odds ratios for DM by serum dioxin decile and maximum serum total lipid decile are calculated using logistic regression analysis. Of the 140 RH diabetics with sufficient data, 27.1% show a temporary increase in serum dioxin and another 20.8% show an unusually slow half life (> 15.5 years). The greatest dioxin increases occur in predominantly obese individuals with higher fasting blood glucose, younger age at DM onset, and greater weight loss with onset. The three lowest RH deciles show significantly lower DM risk versus Comparisons, and the top three deciles show generally younger age distribution and higher rates of obesity at tour and decades later. Significant DM risks in the highest RH decile are attenuated by adjustment for these temporary dioxin increases (substituting second highest value). These RH findings on DM are consistent with the reverse causation hypothesis, where temporarily elevated serum dioxin levels may create an artifactual dose-risk relationship.

PS 2256 UNUSUALLY HIGH LEVELS OF BISPHENOL-A IN CASH RECEIPTS.

R. C. Achuthan[1], S. Babu[1], S. N. Uppu[3], K. Smith[1], N. L. Parinandi[2] and R. M. Uppu[1]. [1]*Environmental Toxicology, Southern University, Baton Rouge, LA,* [2]*DHLRI, The Ohio State University Medical Center, Columbus, OH and* [3]*Prairieville Middle, Prairieville, LA.*

Bisphenol A (BPA) ranks among the top 2% of high volume chemicals produced in the U.S. It is used in the making of printing materials, polycarbonate plastics and resins all of which can become portals for human exposure. The impact of BPA on human health has been debated with toxic effects of BPA becoming evident at doses below 0.05 μg/kg body wt (currently accepted daily intake limit). The issue of health impact of BPA is compounded further by reports showing that contact with thermal paper used at cash registers can be a significant source of BPA exposure and absorption through the skin. In the present study, we collected 34 cash receipts from grocery stores, fast-food restaurants, and banking sectors and analyzed the content of BPA by reversed-phase (RP) HPLC coupled with UV-Vis detection. The analysis involved overnight extraction of BPA in cash receipts with acetonitrile (AN) at 50°C, followed by separation on a Supelcosil LC18 column (4.6 x 150 mm; 5 μ) using 50% AN in water as mobile phase at a flow rate of 1 mL/min (inj. vol.: 20 μL). Under these conditions BPA in cash receipts was found to elute at 3.7 min and showed identical fluorescent properties as that of standard BPA. The identity of BPA in cash receipts was further confirmed by co-chromatography (RP-HPLC) with authentic BPA. When monitored at 232 nm, the RP-HPLC method gave linear response in the concentration range of 1-200 μM BPA. Among the cash receipts analyzed, 41% had BPA in the range of 0.1-9.9 mg/g, 34% contained substantially higher levels (10-20 mg/g) and the reminder 25% did not show the presence of BPA. Considering the fact that Food and Drug Administration has voiced concerns about BPA effects in children and infants and that Environmental Protection Agency is currently developing an action plan for minimizing exposure to BPA, the 100-1,000-fold higher levels of BPA in certain cash receipts merit immediate attention and warrants further investigation. [Corresponding author's email: rao_uppu@subr.edu].

PS 2257 PHARMACOKINETICS, TISSUE DISTRIBUTION, EXCRETION BALANCE, AND METABOLITE PROFILING FOLLOWING A SINGLE INTRAVENOUS OR ORAL DOSE OF [^{14}C]-FORODESINE IN RATS.

D. Draganov[1], D. Sved[1], L. Harman[2] and B. S. Levine[3]. [1]*Metabolism, WIL Research Laboratories, LLC, Ashland, OH,* [2]*BioCryst Pharmaceuticals, Inc., Durham, NC and* [3]*Levine & Associates, LLC, Chicago, IL.*

Forodesine (BCX-1777) is a rationally designed, potent transition-state analog inhibitor of the enzyme purine nucleoside phosphorylase (PNP) currently being evaluated in patients with hematologic malignances. This GLP study determined the pharmacokinetics, tissue distribution, mass balance, and metabolite profile of [^{14}C]-forodesine after a single intravenous (IV, 10 mg/kg, 200 μCi/kg) or oral (100 mg/kg, 200 μCi/kg) dose of [^{14}C]-forodesine in male (M), non-pregnant female (F) and pregnant female (PF; dosed on gestation day 6) Sprague-Dawley rats. Following IV dosing, pharmacokinetic parameters for forodesine equivalents were comparable among individual animals, between the genders, and between F and PF rats. Terminal $T_{1/2}$ was 19, 16.2, and 13.3 h for M, F, and PF, respectively. The apparent V_d (>7 L/kg) indicated extensive tissue binding of the drug (PNP inhibitors bind tightly to the enzyme in tissues and RBCs). Tissue distribution of forodesine equivalents was evaluated by quantitative whole body autoradiography. The concentration of forodesine equivalents in most tissues was highest at 2 h post-dosing (first evaluation time) and then gradually decreased through 24 or 72 h post-dosing. Following IV dosing, ≈90% of the dose was accounted for in urine and cage rinse; total recovery in excreta was >93%. Oral (gavage) administration of [^{14}C]-forodesine resulted in limited systemic exposure to forodesine equivalents; oral bioavailability was ≈5% for M, F, and PF rats. T_{max} was at 2 h post-dosing. Following oral dosing, ≈90% of the dose was accounted for in feces; total recovery in excreta was >94%. Radio-HPLC and LC-MS/MS analysis of plasma pools following IV or oral administration detected only parent drug indicating that forodesine was not metabolized in M, F, and PF rats under the conditions of this study. In summary, forodesine was eliminated in the urine (IV) or feces (PO), and was not metabolized prior to excretion.

PS 2258 PHARMACOKINETICS, EXCRETION BALANCE, AND METABOLITE PROFILING FOLLOWING A SINGLE INTRAVENOUS OR ORAL DOSE OF [^{14}C]-FORODESINE IN CYNOMOLGUS MONKEYS.

B. S. Levine[1], D. Sved[2], L. Harman[3] and D. Draganov[2]. [1]*Levine & Associates, LLC, Chicago, IL,* [2]*Metabolism, WIL Research Laboratories, LLC, Ashland, OH and* [3]*BioCryst Pharmaceuticals, Inc., Durham, NC.*

Forodesine (BCX-1777) is a rationally designed, potent transition-state analog inhibitor of the enzyme purine nucleoside phosphorylase (PNP) currently being evaluated in patients with hematologic malignances. This GLP study determined the pharmacokinetics, excretion balance, and metabolite profile of [^{14}C]-forodesine after a single intravenous (IV, 10 mg/kg, 75 μCi/kg) or oral (100 mg/kg, 25 μCi/kg) dose of [^{14}C]-forodesine in male and female Cynomolgus monkeys. Following IV dosing, plasma and whole blood (WB) concentrations of forodesine equivalents were comparable among the individual animals and between the genders. Forodesine equivalents were eliminated faster from plasma than from WB as demonstrated by ≈3-fold higher AUC_{last} value for WB than for plasma, and an order of magnitude longer MRT_{last} for forodesine equivalents in WB than in plasma. The apparent V_d for forodesine equivalents in plasma (generally >45 L/kg) indicated extensive tissue binding of the drug (PNP inhibitors bind tightly to the enzyme in tissues and RBCs). Following IV dosing, the majority of the administered dose was eliminated in urine; the total recovery in excreta was ≈60% for males and ≈80% for females. Oral (nasogastric) administration of [^{14}C] forodesine resulted in oral bioavailability between ≈ 7% and 10%. . Plasma and WB concentrations of forodesine equivalents were comparable among the individual animals and between the genders. T_{max} was at 1 or 2 h post-dosing. Following oral dosing, forodesine equivalents were eliminated faster from plasma than from WB; the majority of the administered dose (≈ 80%) was eliminated in feces and total recovery in excreta was ≈90%. Terminal $t_{1/2}$ could only be determined for 1 animal (PO; 8.3 hours). Radio-profiling and LC-MS/MS analysis of plasma following IV or oral administration detected only parent drug. In summary, forodesine was eliminated in urine (IV) or feces (PO), and was not metabolized prior to excretion.

PS 2259 INHALATION KINETICS OF 1, 1-DIFLUOROETHANE IN HUMANS.

L. Ernstgård[1], W. Dekant[2], S. Juran[1], B. Lind[1], B. Sjögren[1] and G. Johanson[1]. [1]*Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and* [2]*University of Wuerzburg, Wuerzburg, Germany.*

1,1-difluoroethane (HFC152a) is one of the hydrofluorocarbons (HFCs) replacing the ozone-depleting chlorofluoro- (CFC) and hydrochlorofluorocarbons (HCFC). Human kinetic data on HFCs, and in particular on HFC152a, are sparse. Healthy volunteers of each sex were exposed in random order to 0, 200 or 1000 ppm HFC152a for 2 h during light physical exercise (50 W) in an exposure chamber. Capillary blood, urine and exhaled air were sampled up to 22 h post-exposure and analyzed for HFC152a by head space gas chromatography. Urinary fluoride was analyzed by ion selective electrode. Fluorine-19 NMR analysis was used to identify other possible metabolites in urine. Symptoms of irritation and central nervous system effects were rated in Visual Analogue Scales (VAS). The initial increase of HFC152a in blood was fast and an apparent steady-state was reached within a few minutes at both exposure levels. The post-exposure decrease in blood was equally fast and parallel to that in exhaled air. Only minor amounts of unchanged HFC152a were excreted in breath (2.2 % of inhaled) and urine (0.005%) after exposure. The observed time courses in blood and breath agreed well with those obtained with a physiologically-based pharmacokinetic (PBPK) model. The PBPK simulations indicate a relative uptake during exposure of 2.1%. The cumulative excretion of fluoride in urine was significantly higher after exposure to 1000 ppm compared to control and 200 ppm exposure. No other urinary metabolites were detected. The VAS ratings revealed no HFC152a -related effects. In conclusion, the present study describes for the first time the toxicokinetic behavior of HFC152a in humans. The kinetic profile is similar to what we have previously described for three related hydrofluorocarbons, HFC134a, HFC143a, and HFC245fa. The profiles are proportional to the exposure concentrations, suggesting linear, first-order kinetics at least up to 1000 ppm.

PS 2260 TOXICOKINETICS OF FOLPET BIOMARKERS IN ORALLY AND DERMALLY EXPOSED VOLUNTEERS.

A. Berthet[1, 2], M. Bouchard[1] and B. Danuser[2]. [1]*Department Environmental & Occupational Health, University of Montreal, Montreal, QC, Canada and* [2]*Institute for Work and Health, Lausanne, Switzerland.*

To assess exposure to folpet, a fungicide used to treat crops, biomonitoring appears as a useful tool, but human toxicokinetic data are lacking to help interpret results. According to animal data, folpet is broken down to phthalimide (PI) once absorbed, which is in turn rapidly hydrolyzed to phthalamic acid and phthalic acid (PA). This study aimed at documenting the human kinetics of folpet ring-metabolites in biological matrices of volunteers orally and dermally exposed. Five young male adults were orally exposed to 1 mg/kg of folpet and, two weeks later, were dermally applied 10 mg/kg of folpet. Blood was withdrawn at fixed time periods over the 72 h post-dosing and complete urine voids were collected over 96 h. PI was then quantified in urine and plasma and PA in urine. In plasma, peak levels of PI were observed on average 6 and 10 h following oral and dermal exposure, respectively, and subsequent elimination phase appeared monophasic with respective mean elimination t½ of 31.5 and 29.7 h. Similar elimination t½ of PI were calculated from urinary excretion rate time courses (27.3 and 28.8 h, respectively). Excretion rate time course of PA rapidly evolved in parallel with that of PI following both oral and dermal exposure, and respective t½ of 27.6 and 29.6 h were calculated. The percentage of dose recovered as PI in urine was 0.025 and 0.002%, respectively, after oral and dermal dosing; corresponding values for PA were 25 and 1.8%. PI and PA were thus rapidly formed and eliminated following both oral and dermal exposure. Although these two biomarkers exhibited similar time profiles, PA was present in much higher amounts than PI in urine, in line with the substantial metabolism of PI into acids. Results also show a negligible effect of the dermal absorption rate on elimination rate of PI and PA from the body. However, the ≈10-fold lower percentage of dose found as PI or PA in urine after dermal exposure compared to oral administration indicates a low dermal absorption fraction.

PS 2261 TISSUE DISTRIBUTION AND EXCRETION OF SR13668 IN RATS.

J. M. Gow[1], K. O'Loughlin[1], A. Ta[1], L. Jong[1], I. M. Kapetanovic[2] and C. E. Green[1]. [1]*Biosciences Division, SRI International, Menlo Park, CA and* [2]*National Cancer Institute, Bethesda, MD.*

Lead-based drug design identified SR13668 as a potent analog of indole-3-carbinol with enhanced in vitro and in vivo anticancer effects and decreased toxicity. Pharmacokinetics studies of SR13668 in rat, dog and monkey demonstrated acceptable oral bioavailability and drug levels concentrated in lung. To evaluate excretion and tissue distribution, ^{14}C-SR13668 was synthesized and administered to Sprague-Dawley rats by intravenous (iv; DMSO:PEG300, 15:85) and oral gavage (po; PEG400:Labrasol, 1:1) dose routes. Feces and urine were collected from rats at time points through 120 hr. Total radioactivity was determined by liquid scintillation counting. The majority of the dose, administered either iv or po, was recovered in the feces by 48 hr. Major organs (adipose, brain, heart, intestine, kidney, liver, lung, skeletal muscle, skin and stomach) were harvested at 6 and 24 hr post-dose and analyzed for total radioactivity. Blood and plasma were collected at time points through 24 hours. After iv administration (1 mg/kg), lung contained ~13% of dose administered or 40-fold greater than plasma levels at 6 and 24 hr. Liver also contained a high fraction of the dose, ~5% at 6 hr and a wide range (5-67%) of radioactivity was recovered in the intestine, suggesting that SR13668 undergoes hepatic elimination. After po administration (30 mg/kg), the majority of the dose was in the stomach and intestine at 6 hr, with still elevated levels in the intestine at 24 hr. Radioactivity levels in lung and liver were 6 to 11-fold and 12 to 14-fold greater than plasma, respectively. In summary, the primary route of excretion of total radioactivity from ^{14}C-SR13668 was via the feces with the majority of the dose recovered within 48 hours. Hepatic metabolism is likely to be involved in elimination of SR13668 and this was supported by the tissue distribution data of high levels in the intestine and liver after both the iv and po routes. Consistent with previous studies, SR13668 also concentrates in the lung. (Supported by NCI Contract HHSSN261200433005C)

PS 2262 PHARMACOKINETICS OF A PYRETHROID INSECTICIDE MIXTURE IN THE RAT.

D. G. Ross[1], J. M. Starr[2], E. J. Scollon[3], K. M. Crofton[1], M. J. Wolansky[4], M. F. Hughes[1] and M. J. DeVito[5]. [1]*ORD/NHEERL, U.S. EPA, Research Triangle Park, NC,* [2]*ORD/NERL, U.S. EPA, Research Triangle Park, NC,* [3]*OCSPP/HED, U.S. EPA, Alexandria, VA,* [4]*University of Buenos Aires, Buenos Aires, Argentina and* [5]*NTP, NIEHS, Research Triangle Park, NC.*

Pyrethroid insecticides are used and co-occur in the environment, in residences and day care facilities. Pyrethroids have a similar mode of action. Pharmacokinetic models of pyrethroids and assessment of risk from their exposure would be better informed if data are derived from studies using chemical mixtures. The objective of this study was to assess the pharmacokinetics of a pyrethroid mixture. The pyrethroids esfenvalerate (3% of mixture), deltamethrin (3%), cyfluthrin (13%), cis-permethrin (21%), cypermethrin (29%) and trans-permethrin (31%) were administered po (corn oil, 1 ml/kg) to adult male Long Evans rats. The doses were based on the distribution of pyrethroid residues measured in a nationally representative probability study of child care centers (Tulve et al., Environ Sci Technol,

2006, 40:6269-74). Rats received either a low (11.2 mg/kg) or high (27.4 mg/kg) pyrethroid mixture dose and were sacrificed at 1, 2, 4, 8 or 24 h after dosing. Blood, liver, fat and brain were removed. Tissues were extracted and analyzed for parent chemical by LC/MS/MS. For both doses, pyrethroid residues were greater in fat relative to blood, brain and liver, based on area under the curve from 0 to 24 h (AUC_{0-24h}) and maximal concentration (C_{max}). With dose, the AUC_{0-24h} increased 4- to 9-fold in blood, 2- to 3-fold in brain, 2-fold in fat and 4- to 6-fold in liver. There were similar increases in pyrethroid tissue C_{max} with dose. Cypermethrin and cis-permethrin consistently had the highest AUC_{0-24h} and C_{max} in all tissues. trans-Permethrin consistently had lower AUC_{0-24h} and C_{max} in all tissues, which may be due to increased metabolism relative to the other pyrethroids. Determining the pharmacokinetics of a mixture of environmental chemicals such as the pyrethroids is important when assessing the risk to chemicals that have a similar mode of action. (This abstract does not represent US EPA policy.)

PS 2263 EVALUATING PHARMACOKINETICS AND BIOAVAILABILITY OF LEVOFLOXACIN IN RATS.

A. Banerjee[1], K. Kabirov[1], T. Martín-Jiménez[2] and A. Lyubimov[1]. [1]*Toxicology Research Laboratory, University of Illinois at Chicago, Chicago, IL and* [2]*College of Veterinary Medicine, University of Tennessee, Knoxville, TN.*

Drug exposure of the antibiotic levofloxacin was examined in Sprague Dawley rats following oral and intravenous administration. Six female and six male cannulated rats were randomly assigned to two treatment groups (3 males + 3 females/ group). One group received a single IV dose of levofloxacin at 25 mg/kg and the other received a single oral dose at 50 mg/kg. Blood samples were collected, analyzed and the data used for pharmacokinetic analysis. Intravenous pharmacokinetic profiles were very similar in males and females, with the exception of systemic clearance, which was 2222 and 2667 ml/hr/kg in males and females, respectively. Accordingly, the AUC0-inf values were higher in males (11264 vs. 9464). The estimated values of C0, Cmax, Vss and terminal elimination half-life were very similar across genders. The volume of distribution was rather large (Vss: 3.2-3.6 L/kg), which indicates wide distribution and high affinity of the drug for proteins and perhaps other molecules in tissue. The absolute oral bioavailability of levofloxacin in rats was 34% in males and 47% in females which is relevant for a concentration-dependent antibiotic such as levofloxacin. Accordingly, the AUC0-inf after the oral administration was lower in males than in females (7668 and 8952 ng*hr/mL, respectively). The larger gender-related difference in oral pharmacokinetics was in Cmax and average values in males and females were 1370 and 2733 ng/mL, respectively. Coefficients of variability for this parameter were rather high (43-59%). The half-life of the drug after intravenous administration was short (1.5-2.0 hr) but it increased after oral administration (3.9-4.1 hr). Mean terminal elimination half-lives were very similar in males and females (3.9 and 4.1, respectively). In conclusion, bioavailability seen in rats is about 2-fold lower than in humans indicating species based differences in absorption profile of levofloxacin.

PS 2264 ROLE OF INTESTINAL P450 AND P-GLYCOPROTEIN IN MODULATING THE BIOAVAILABILITY OF ORAL LOVASTATIN.

Y. Zhu. *Environmental Health Sciences, Wadsworth Center, New York State Department of Health, and School of Public Health, State University of New York at Albany, Albany, NY.* Sponsor: X. Ding.

Variations in drug metabolism and transport can affect drug bioavailability, and potentially cause drug toxicity. However, the roles of intestinal enzymes or drug transporters in controlling the bioavailability of oral drugs are not well defined for many drugs. Our aim was to determine the specific roles of small intestinal (SI) P450 enzymes and the P-glycoprotein (P-gp) in the first-pass clearance of lovastatin (LVS), an anticholesterol drug. In an intestinal epithelium (IE)-specific P450 reductase (CPR) knockout (IE-Cpr-null) mouse model, which has little P450 activities in the IE, we found that the rates of LVS metabolism by SI microsomes were reduced by >90%, compared with wild-type (WT) mice. In vivo clearance of oral LVS was much slower in IE-Cpr-null than in WT mice; consequently, the bioavailability of LVS was 14% in IE-Cpr-null mice, but only 5% in WT mice. In contrast, the clearance rates for intraperitoneally dosed LVS were not different between the two mice strains. These results demonstrate a predominant role of SI P450 enzymes in the first-pass clearance of LVS. This conclusion was further supported by additional data, obtained through pharmacokinetic studies on a liver-specific Cpr knockout (liver-Cpr-null) mouse model, indicating minimal contribution of hepatic P450 enzymes to the first-pass clearance of oral LVS. The specificity of the impact of the CPR loss on SI P450 activity was confirmed by the results from pharmacokinetic studies for pravastatin (PVS), a drug which is similar to LVS, but is not a P450 substrate. We then determined whether P-gp contribute to LVS clearance, by using cyclosporin A (CsA), an inhibitor of both P450 and P-gp, and by taking advantage of the absence of P450 activities in the SI of the IE-Cpr-null mice. We found that CsA treatment (oral) significantly enhanced the systemic exposure of LVS in both IE-Cpr-null and WT mice, a result strongly suggest that P-gp also influences the bioavailability of oral LVS in mice. (Supported in part by NIH grant GM082978)

PS 2265 PARAQUAT DISPOSITION: A POTENTIAL ROLE FOR P-GLYCOPROTEIN TRANSPORT.

S. Lacher, F. Cardozo-Pelaez and E. L. Woodahl. *University of Montana, Missoula, MT.*

P-glycoprotein (P-gp) is an efflux drug transporter expressed in many tissues important in xenobiotic disposition. At the blood brain barrier (BBB), P-gp is one of the mechanisms that protects the brain from toxic substances circulating in the blood. P-gp has been proposed to play a role in the susceptibility to Parkinson's disease (PD), possibly by altering accumulation of neurotoxicants in the brain. However, evidence is missing as to whether neurotoxicants associated with PD are P-gp substrates and if P-gp alters their ability to cross the BBB. Our goal is to measure P-gp transport of neurotoxic xenobiotics associated with PD across the BBB. Our recent studies focus on the pesticide paraquat with the goal of measuring paraquat toxicokinetics, paraquat brain accumulation, and markers of neurodegeneration in FVB mice. We administered paraquat to FVB mice to measure brain accumulation and estimate paraquat toxicokinetics. Two different groups of FVB mice were administered a single oral dose of paraquat (10mg/kg and 25mg/kg), plasma samples were collected at 1, 2, 4, and 8 hours, and the brains were harvested at 8 hours. Plasma and brain samples were analyzed for total paraquat concentration using high performance liquid chromatography. We were able to detect paraquat in the plasma and brain samples in both treatment groups. We found that the average elimination half-lives of the 10mg/kg and the 25mg/kg treatment groups were similar at approximately 8 hours, which suggests that there is no dose effect on half-life. Average brain accumulations were 1.02 +/- 0.21μg and 0.95 +/- 0.44μg after 10mg/kg and 25mg/kg doses, respectively. Our future studies will examine the toxicokinetics of paraquat disposition in P-gp knock-out (mdr1a-/-/mdr1b-/-) mice, as well as identifying markers of neurodegeneration. This study will provide the missing data to measure P-gp-mediated transport of paraquat and the susceptibility to Parkinson's disease.

PS 2266 THE IMPORTANCE OF EFFLUX TRANSPORTERS IN CNS EXPOSURE TO AVERMECTIN INSECTICIDES.

A. M. Dalzell[1], F. M. Williams[2], P. Mistry[3], J. Wright[3] and C. D. Brown[1]. [1]*Cell & Molecular Biosciences, University of Newcastle, Newcastle-Upon-Tyne, United Kingdom,* [2]*Medical Toxicology Centre, University of Newcastle, Newcastle-Upon-Tyne, United Kingdom and* [3]*Syngenta, Bracknell, United Kingdom.* Sponsor: R. Peffer.

Avermectins are used as agricultural insecticides and as anti-helminthic treatments in man. Avermectins have good safety profiles in a variety of species; however neurotoxicities observed in some CF-1 mice initiated investigations into the relevance of this mouse model for human risk assessment. Susceptible CF-1 mice lack the functional xenobiotic efflux transporter, P-glycoprotein (mdr1a/P-gp), demonstrating its importance in neuroprotection. In humans a non-functional MDR1 has yet to be identified, although the importance of MDR1 in xenobiotic efflux in man needs to be better understood. In this study we compared two neuroblastoma cell lines; human SH-SY5Y cells, characterised for uptake and efflux transporter expression, with mouse N2a cells to identify species differences in avermectin handling. Intracellular levels of the fluorescent MDR1-substrate H33342 dye in the presence of avermectins (0-7μM) were compared to the increase ($p<0.05$) in dye retention seen with the model MDR1 substrate cyclosporin A (CSA, 5μM). The MTT assay confirmed cell integrity after avermectin treatment. Incubation of cells with each avermectin (5μM) resulted in an increase ($p<0.05$) in intracellular dye retention, similar to that with CSA. Dose response curves for the ability to inhibit MDR1-mediated H33342 dye efflux gave apparent Ki values of 0.9.±.0.2 v 1.20±0.5μM (abamectin, respectively in human and mouse cells); 0.58±0.1 v 0.50±0.1μM (emamectin benzoate) and 0.28±0.08 v 0.16±0.03μM (ivermectin). These values compared with Ki of 0.91±0.5 v 0.55±0.2μM for CSA interaction with MDR1 and mdr1a. There were no differences in the affinities of individual avermectins for human versus mouse P-gp ($p>0.05$). The apparent rank order of both MDR1 and mdr1a affinities was ivermectin > emamectin > abamectin, in human and mouse cells. Parallel experiments using a CMFDA dye assay suggest that the avermectins may be substrates for MRP efflux transporters expressed in both cell types

PS 2267 SENSITIVE AND SPECIFIC FLUORESCENT PROBES FOR FUNCTIONAL ANALYSIS OF ALL THREE MAJOR TYPES OF MAMMALIAN ABC TRANSPORTERS.

W. F. Patton, I. V. Lebedeva, P. Pande, D. Shen and D. Gatica. *R&D, Enzo Life Sciences, Farmingdale, NY.* Sponsor: J. Davis.

An underlying mechanism for multi drug resistance (MDR) is up-regulation of the transmembrane ATP-binding cassette (ABC) transporter proteins. ABC transporters also determine the general fate and effect of pharmaceutical agents in the body. The three major types of ABC transporters are MDR1 (P-gp, P-glycoprotein, ABCB1), MRP1/2 (ABCC1/2) and BCRP/MXR (ABCG2) proteins. Flow cytometry (FCM) allows determination of the functional expression levels of ABC transporters in live cells, but most dyes used as indicators (Rhodamine 123, DiOC2(3), calcein-AM) have limited applicability as they do not detect all three major types of ABC transporters. Dyes with broad coverage (such as doxorubicin, daunorubicin and mitoxantrone) lack sensitivity due to overall dimness and thus may yield a significant percentage of false negative results. We describe two novel fluorescent probes that are substrates for all three common types of ABC transporters and can serve as indicators of MDR in FCM assays using live cells. The probes exhibit fast internalization, favorable uptake/efflux kinetics and high sensitivity of MDR detection, as established by multidrug resistance activity factor (MAF) values and Kolmogorov-Smirnov statistical analysis. Used in combination with general or specific inhibitors of ABC transporters, both dyes readily identify functional efflux and are capable of detecting small levels of efflux as well as defining the type of multidrug resistance. The assay can be applied to the screening of putative modulators of ABC transporters, facilitating rapid, reproducible, specific and relatively simple functional detection of ABC transporter activity and ready implementation on widely available instrumentation.

PS 2268 OAT2 REPRESENTS AN INITIAL STEP IN THE RENAL TUBULAR SECRETION OF GUANINE-CONTAINING ANTIVIRAL DRUGS.

Y. Cheng and R. Pelis. *DMPK-DDI, Novartis Institutes for BioMedical Research, East Hanover, NJ.*

Guanine-containing antiviral drugs, including acyclovir, ganciclovir and penciclovir, are mainly eliminated in the urine by glomerular filtration and active tubular secretion. The organic anion transporters 1 (OAT1), 2 (OAT2) and 3 (OAT3), and the organic cation transporter 2 (OCT2), are expressed in basolateral membranes of renal proximal tubule cells and represent the initial step (uptake) in the tubular secretion of numerous drugs. The purpose of the present study was to determine if OAT1, OAT2, OAT3 and/or OCT2 are relevant to the renal clearance of acyclovir, ganciclovir and penciclovir. The human orthologs of OAT1, OAT2, OAT3 and OCT2 were stably expressed in the Human Embryonic Kidney (HEK) cells to investigate the uptake of these antiviral drugs in vitro. The uptake of acyclovir, ganciclovir and penciclovir was 10-fold, 12-fold and 20-fold higher, respectively, in hOAT2 expressing HEK cells compared to parental HEK cells. The Km values for hOAT2-mediated acyclovir, ganciclovir and penciclovir transport were 94 μM, 264 μM and 284 μM, respectively. The transport efficiency (Jmax/Km) was ~3-fold higher for penciclovir compared to the other drugs tested. In comparison, active uptake of these antiviral drugs into OAT1, OAT3 and OCT2 expressing HEK cells was weak to absent. Based on these in vitro results, OAT2 is likely the main transporter involved in the active uptake of acyclovir, ganciclovir and penciclovir into renal proximal tubule cells. This is the first study to show that OAT2, compared to OAT1, OAT3 and OCT2, exhibits a preference for drugs mainly excreted into the urine.

PS 2269 COMPARISON OF TRANSPORTER EXPRESSION IN BRAIN, EYE, TESTIS, AND PLACENTA.

F. Selwyn, J. Liu and C. Klaassen. *Pharmacology and Toxicology, University of Kansas Medical Center, Kansas City, KS.*

Transporters play an important role in the absorption, distribution and elimination of endogenous substances as well as xenobiotics. Transporters show tissue specific expression depending on their function. Transporters of the ATP-binding cassette (Abc) superfamily and Solute carrier family are present in the blood-brain barrier and play a protective role in tightly regulating which substances can enter the brain. Other tissues, such as eye, testis and placenta also have barriers. However, it is not known whether the transporters expressed in eye, testis, and placenta are the same as those in brain. Therefore, we quantified the mRNA expression of 30 transporters in eye, testis and placenta of male and female mice using real-time RT-PCR, and compared their expression to that in brain. Ten transporters namely, cholesterol efflux regulatory protein Abca1, multidrug-resistance protein Mdr1a (P-glycoprotein), multidrug resistance-associated proteins Mrp1 and Mrp4, breast cancer resistance protein Bcrp, organic anion transporting polypeptide Oatp1a4, organic cation/carnitine transporter Octn1, Octn2, equilibrative nucleoside transporter Ent1 and aminophospholipid transporter Atp8b1 are highly expressed in brains, eyes, testis and placenta except Oatp1a4, which is not highly expressed in placenta and Octn1, which is not highly expressed in eye. Mdr1a, Mrp1, Mrp4 and Bcrp are xenobiotic efflux transporters, which protect tissues by limiting the tissue concentration of circulating xenobiotics. Abca1, Ent1, Octn1 and Octn2 are nutritive transporters that are highly expressed to provide nutrients to the organ and the fetus. Atp8b1 is important in maintaining membrane integrity by regulating proportion of cholesterol and sphingomyelin in outer leaflet of cell membranes. Therefore, the data suggests that, transporters that are expressed in eye, testis and placenta are similar to those expressed in the brain. These transporters together, could be integral components of tissue-blood barriers. (Supported by the grants RR021940, RR016475, ES009649, ES013714, ES007079, DK081461)

PS 2270 DIFFERENTIAL HEPATIC AND INTESTINAL PHASE-II BIOTRANSFORMATION ENZYME/TRANSPORTER EXPRESSION AND SERUM BISPHENOL A (BPA) LEVELS IN MOUSE MODELS OF OBESITY AND DIABETES.

O. DiPrete[1], V. R. More[1], J. Xu[1], D. Doerge[2] and A. Slitt[1]. [1]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI and* [2]*Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. FDA, Jefferson, AR.*

Approximately 7.8% of Americans have Diabetes Mellitus. Other diseases also often co-present with diabetes, such as non-alcoholic fatty liver disease (NAFLD). Diet-induced obesity and NAFLD affect hepatic transporter expression in mice, but it is not known whether these conditions affect intestine transporter expression. Moreover, little information exists regarding whether obesity/diabetes affects processes that absorb and metabolize environmental chemicals, such as Bisphenol A (BPA). BPA can leach out of plastics, and BPA exposure might be associated with adverse health effects. Duodenal transporter and hepatic UDP-glucuronosyl transferase (UGT) mRNA expression of Leprdb/db (db/db) mice and diet-induced obese mice (DIO) was determined. A single D16-BPA dose (100 mg/kg, saline, po) was administered to male C57BKS, db/db, C57BL/6, or DIO mice. In the duodenum of male db/db mice, Abcg2 mRNA expression was decreased, whereas Abcb1 and Abcc3 mRNA expression was increased, as compared to C57BKS mice. Abcg2 mRNA expression was increased, whereas Abcb1 mRNA expression was decreased in duodenum of DIO mice compared to lean mice. Ugt1a1, 1a6, 2a3, 2b1, 2b5, and 3a2 mRNA expression was decreased in livers of db/db females compared to C57BKS females. In db/db males, Ugt2b1 and 2b5 mRNA expression in liver was decreased compared to C57BKS males. In contrast, DIO increased Ugt1a1, 3a1 and 3a2 mRNA expression in liver compared to lean controls. In db/db mice, serum BPA (aglycone) concentrations were 5-fold higher than C57BKS mice, but conjugated BPA and total BPA levels were equivalent, suggesting decreased glucuronidation. In DIO mice, total BPA, BPA (aglycone), and conjugated BPA levels were increased, suggesting possible differences in BPA absorption and glucuronidation (NIH 3R01ES016042-02S1).

PS 2271 TOXICOKINETICS OF PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANE SULFONATE (PFOS) AFTER A SINGLE INTRAVENOUS OR GAVAGE ADMINISTRATION TO HARLAN SPRAGUE-DAWLEY RATS.

S. Hong[1], V. Robinson[2], S. Gibbs[1], S. W. Graves[1], B. Burback[1], M. Eifrid[1] and C. Smith[2]. [1]*Battelle Memorial, Columbus, OH and* [2]*NIEHS, NIH, Research Triangle Park, NC.*

PFOA and PFOS are considered as global contaminants since these compounds are detected in humans and wildlife across various geographical locations. This study determined the concentrations of PFOA and PFOS in rat plasma. Groups of male and female Sprague Dawley rats (8 to 9 weeks old) were given a single IV or gavage administration of PFOA or PFOS in 2% Tween 80 in deionized water at dosages of 6 (M/IV), 40 (F/IV), 6, 12, 48 (M/gavage), or 40, 80, 320 mg/kg (F/gavage) of PFOA and 2 (IV) or 2, 20 mg/kg (gavage) of PFOS. Concentration-time profiles for PFOA and PFOS were characterized by compartmental modeling using WinNonlin (v 5.0.1). There were gender-related differences in TK parameters for PFOA but not for PFOS. After IV administration, male and female elimination half-life values were 153 and 2.23 hours for PFOA and 528 and 552 hours for PFOS, respectively.

Male and female AUC_∞ values were 11.6 and 1.19 mg/mL*hr for PFOA and 3.66 and 5.36 mg/mL*hr for PFOS, respectively, after IV administration. After gavage administration, elimination half-life values ranged from 215 to 300 hours in males and from 2.75 to 13.9 hours in females for PFOA while values for PFOS were comparable ranging from 457 to 682 hours for both males and females. PFOA AUC_∞ values increased in a nearly dose-proportional manner from 16.3 to 73.9 and from 2.16 to 23.6 mg/mL*hr for males and females, respectively. PFOS AUC_∞ values increased in a nearly dose-proportional manner from 4.93 to 74.9 and from 8.87 to 107 mg/mL*hr for males and females, respectively. The results of this study provided detailed TK parameters for PFOA and PFOS in rats to correlate toxic effects with systemic availability and to improve the usefulness of toxicity study results in risk assessment. This work was supported by NIH (N01-ES-55551).

PS 2272 TOXICOKINETICS OF PERFLUOROHEXANOIC ACID (PFHXA) AND PERFLUOROHEXANE-1-SULPHONIC ACID POTASSIUM SALT (PFHSKSLT) AFTER A SINGLE INTRAVENOUS OR GAVAGE ADMINISTRATION TO HARLAN SPRAGUE-DAWLEY RATS.

S. Gibbs[1], V. Robinson[2], S. Hong[1], S. W. Graves[1], B. Burback[1], C. Granville[1], M. Eifrid[1] and C. Smith[2]. *[1]Battelle Memorial, Columbus, OH and [2]NIEHS, NIH, Research Triangle Park, NC.*

PFHxA and PFHSKslt are considered as global contaminants due to detection in humans and wildlife across various geographical locations. This study determined the toxicokinetics of PFHxA and PFHSKslt in rat plasma. Groups of male and female Sprague Dawley rats (7 to 9 weeks old) were given a single IV or gavage administration of PFHxA or PFHSKslt in 2% Tween 80 in deionized water at dosages of 40 (IV), 40, 80, 160 (gavage) of PFHxA and 4 (IV) or 4, 16, 32 mg/kg (gavage) of PFHSKslt. Concentration-time profiles for PFHxA and PFHSKslt were characterized by compartmental modeling using WinNonlin (v 5.0.1). There were gender-related differences in TK parameters for PFHxA and PFHSKslt. After IV administration, male and female elimination half-life values were 0.812 and 0.346 hours for PFHxA and 311 and 16.8 hours for PFHSKslt, respectively. Male and female AUC_∞ values were 0.295 and 0.122 mg/mL*hr for PFHxA and 20.3 and 1.46 mg/mL*hr for PFHSKslt, respectively, after IV administration. After gavage administration, elimination half-life values ranged from 1.63 to 2.87 hours in males and from 1.11 to 1.38 hours in females for PFHxA, and from 354 to 423 hours in males and from 47.6 to 55.9 hours in females for PFHSKslt. PFHxA AUC_∞ values increased in a nearly dose proportional manner from 0.387 to 1.09 and from 0.244 to 0.584 mg/mL*hr for males and females, respectively. PFHSKslt AUC_∞ values increased in a slightly less than dose proportional manner from 19.9 to 85.1 and from 2.08 to 8.34 mg/mL*hr for males and females, respectively. The results of this study provided TK parameters for PFHxA and PFHSKslt in rats to correlate toxic effects with systemic availability and to improve the usefulness of toxicity study results in risk assessment. This work was supported by NIH (N01-ES-55551).

PS 2273 ABSORPTION AND EXCRETION OF ^{14}C-PERFLUOROOCTANOIC ACID (PFOA) IN BEEF CATTLE.

J. Huwe[1], S. Lupton[1], D. J. Smith[1], K. Dearfield[2] and J. J. Johnston[3]. *[1]USDA, ARS, Biosciences Research Lab., Fargo, ND, [2]USDA, FSIS, Office of Public Health Science, Washington, DC and [3]USDA, FSIS, Office of Public Health Science, Fort Collins, CO.*

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are industrial chemicals that are environmentally persistent. PFOS has recently been classified as a persistent organic pollutant under the Stockholm Convention. Both PFOS and PFOA can be found in biosolids, and the application of contaminated biosolids to pastures used for beef cattle production has raised concerns about the possible accumulation of PFOA and PFOS in the edible tissues of these animals. As such, the United States Department of Agriculture (USDA) has undertaken a study to determine the absorption, distribution, metabolism, and excretion of PFOA and PFOS in beef cattle following an oral dose. The animal protocol was approved by the local Institutional Animal Care and Use Committee and USDA Radiation Safety Committee. Four Lowline Angus steers (281–366 kg) were given single oral bolus doses containing ^{14}C-PFOA (1 mg/kg) and unlabeled PFOS (10 mg/kg). Serum, plasma, urine, and feces were collected prior to and after dosing at various intervals from each steer for 28 days. Radioactivity in the serum, plasma, and urine was determined by liquid scintillation counting (LSC) and in the feces by combustion analysis followed by LSC. Radioactivity derived from ^{14}C-PFOA was completely absorbed and excreted via the urine within 8 days of dosing (100.6 ± 3.3%). Blood and urine levels peaked between 24 and 36 hrs post-dose, and the serum elimination half life was 24 hrs. Minimal amounts of radioactivity were excreted in the feces (2–3%). Studies in rodents and non-human primates have calculated PFOA serum half lives of 10–30 d. One exception is female rats which have markedly increased urinary excretion rates and half lives of 3–5 hr. This study shows that PFOA is rapidly excreted by steers and indicates that it would be unlikely to accumulate in the edible tissues. Samples from this study have not yet been analyzed for PFOS.

PS 2274 SPECIES-SPECIFIC FACTORS WHEN PREDICTING SYSTEMIC CLEARANCE OF ENB-0040 IN RABBITS *VIA* AN ALLOMETIC SCALING APPROACH.

C. Jomphe[1], I. Lemire[2], P. Leonard[2], M. Reimer[1] and M. Beliveau[1]. *[1]RAS, Pharsight Corporation, Montreal, QC, Canada and [2]Enobia Pharmacology Montreal, QC, Canada.*

ENB-0040 is a soluble form of tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) developed by Enobia Pharma for the treatment of hypophosphatasia, a rare metabolic bone disease. The objective of this study was to estimate clearance (CL) of ENB-0040 in rabbits using a previously developed allometric scaling approach of available PK data. The CL of ENB-0040 for intravenous (IV) administration in rabbit was evaluated according to body weight (BW) and the following equation: $CL = (1.31\times10^{-5}) \times BW^{0.929}$ in WinNonlin®. This equation was previously developed and validated using mouse, rat, dog, monkey and human PK data. The estimation of ENB-0040 CL following IV dosing was performed using typical rabbit body weight values of 4 kg. The predicted CL value was 0.0291 L/h, which was approximately 10 times lower than the mean experimental non-compartmental CL value of 0.2 L/h obtained from 12 rabbits with WinNonlin. This discrepancy was investigated further by comparing these predicted CL rabbit values to predicted CL value in monkeys, an animal with a similar BW (3 kg), using the same allometric model. The predicted CL in monkey was 0.0223L/h, which compared well to the experimental non-compartmental analysis value of 0.051 L/h in this animal species. This result suggests a potential species-specific factor when predicting CL in rabbit; BW allows for appropriate prediction in monkeys but not rabbits, although BW's are similar between the two species. The results presented in this study highlight some of the potential limitations when using allometric scaling approaches across a wide range of animal species.

PS 2275 METABOLISM AND DISPOSITION OF 2-METHOXY-4-NITROANILINE IN MALE AND FEMALE SPRAGUE-DAWLEY RATS AND B6C3F1 MICE.

S. Waidyanatha[1], Q. Zhan[2], I. Surh[1], S. Warren[2], P. Patel[2], S. Black[2], J. Burgess[2] and J. Mathews[2]. *[1]National Institute of Environmental Health Sciences, Research Triangle Park, NC and [2]RTI International, Research Triangle Park, NC.*

2-Methoxy-4-nitroaniline (MNA) is used in dyeing textiles, as a dye in the printing industry, and as an intermediate in the synthesis of azo dyes that have applications in tattoo inks, emulsion paints, and toy enamels. MNA has structural similarity to the known carcinogens, 2-methoxy-5-nitroaniline (5-nitro-o-anisidine), o-anisidine and 2,4-diaminoanisole. Limited ADME data is available for MNA, and preliminary reports of its metabolism indicated that O-demethylation and nitroreduction followed by N-acetylation were primary metabolic pathways. The present studies were conducted to investigate the metabolism and disposition of dermally and orally applied MNA. Ring-labeled [14C]MNA was administered po to male and female Harlan Sprague Dawley (HSD) rats at 2, 15, and 150 mg/kg, and B6C3F1 mice at 15 mg/kg, and dermally to male HSD rats and B6C3F1 mice at 15 mg/kg; urine and feces were collected for up to 72 h post dosing and tissues were sampled at either 24 or 72 h post doing and analyzed for radiochemical content. Urinary metabolites were profiled by HPLC radiochromatography, and characterized by LC/MS and NMR. In both rats and mice, oral doses of MNA were well absorbed (>75%), and excreted mostly in urine, and only ≤1% of the radioactivity remained in tissues at 24 h or 72 h post dosing. MNA was poorly absorbed following dermal administration, with 5-6% and ca. 10% of the dose systemically absorbed in rats and mice, respectively, in 24 h. The primary route of metabolism in rats was via formation of 6-OH-2-methoxy-4-nitroaniline, followed by O-sulfation to form the major metabolite (characterized by LC/MS and 1H-NMR), as well as O-glucuronidation. The conversion of MNA to its 6-hydroxy metabolite may present a potential for alkylation of macromolecules via oxidation to a reactive iminoquinone.

PS 2276 METABOLISM AND DISPOSITION OF N-BUTYLPARABEN FOLLOWING ORAL AND DERMAL ADMINISTRATION.

J. Mathews[1], S. Black[1], C. Blystone[2], S. Brown[1], P. Patel[1], A. Etheridge[1] and S. Waidyanatha[2]. *[1]RTI International, Research Triangle Park, NC and [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

n-Butylparaben (BPB) belongs to a class of parabens widely used as antioxidants and preservatives in foods, pharmaceuticals, and cosmetics. Limited ADME data has been report for BPB, and metabolites monitored for exposure assessment have been limited to those from hydrolysis of the butyl ester common to the class of parabens and sulfation/glucuronidation of the phenolic group. The present studies were conducted to investigate the absorption and disposition of dermally and orally applied BPB, and to determine its metabolites, particularly those specific to BPB itself. Ring-labeled [14C]BPB was administered to male and female Harlan Sprague Dawley rats po at 10, 100, or 1000 mg/kg, and to male rats dermally at 10 and 100 mg/kg; urine and feces were collected for up to 72 h post dosing and tissues were sampled at either 24 or 72 h post doing and analyzed for radiochemical content. Urinary metabolites were profiled by HPLC radiochromatography, and characterized by LC/MS. Oral doses of BPB were well-absorbed (>83%) and eliminated chiefly in urine (83-84%), and only ≤1% of the radioactivity remained in tissues at 24 h (100 mg/kg) or 72 h post dosing. At the highest dermally applied dose (100 mg/kg), BPB was poorly absorbed (4% at 24 h, 8% at 72 h), but 50% of a 10 mg/kg dose was absorbed in 72 h. Metabolites detected in urine included those previously reported, BPB glucuronide, BPB sulfate, and hydroxyhippuric acid, but also novel catechol metabolites arising from ring hydroxylation followed by glucuronidation and sulfation. This work identified novel BPB-specific, potentially hormonally active metabolites, which have not been identified for this class of possible reproductive toxicants.

PS 2277 DIMETHYLETHANOLAMINE INCREASED SERUM CHOLINE, BUT DID NOT ALTER [14C]-CHOLINE TISSUE DISTRIBUTION IN WISTAR HAN RATS.

J. M. Sanders[2], C. J. Wegerski[1], M. Doyle-Eisele[1] and J. D. McDonald[1]. *[1]LRRI, Albuquerque, NM and [2]NTP/NIEHS, Reserach Triangle Park, NC.*

Dimethylethanolamine (DMAE) is a high volume chemical used in industrial and consumer products, including dietary supplements. DMAE is structurally similar to the essential nutrient choline; therefore, in vivo exposure to DMAE may interfere with choline disposition and metabolism. The present studies investigated interactions of DMAE and choline by determining the effect of DMAE on the disposition of [14C]-choline in male and female Wistar Han rats and by assessing the incorporation of DMAE-derived 14C in tissue phospholipids and the effect of DMAE on serum choline levels in male rats. In the disposition studies, male and female rats received single doses of 100 or 500 mg DMAE/kg or male rats received three daily doses of 100 mg DMAE/kg by gavage prior to oral administration of 160 mg/kg of [14C]-choline. Approximately 25 % of choline-derived 14C remained in tissues 24 h after dosing, and these concentrations were not affected by DMAE pretreatment. Similar amounts of 14C remained in tissues of rats 24 h after gavage of up to 500 mg/kg of [14C]-DMAE only. DMAE-derived 14C was incorporated into tissue phospholipids of male rats, especially in the lung, kidney and liver. The effect of DMAE on endogenous serum choline levels was investigated in jugular vein cannulated male rats gavaged with 500 mg DMAE/kg. Serum was analyzed by LC-MS/MS for choline concentrations prior to and at 1,2, 6, and 12 h after dosing. Serum choline concentrations increased approximately 30 % initially, and remained elevated up to 6 h. In conclusion, DMAE was well absorbed following oral administration to rats and DMAE-derived 14C persisted in tissues over time due to incorporation into membrane phospholipids. Although DMAE administration resulted in increased levels of serum choline, DMAE did not appear to compete directly with incorporation of choline-derived-14C in tissues within the dosing range used in these studies. (This work was conducted under NIEHS contract N01-ES-75562).

PS 2278 TOXICOKINETICS OF CAPTAN BIOMARKERS IN ORALLY AND DERMALLY EXPOSED VOLUNTEERS.

M. Bouchard[1], A. Berthet[1, 2] and D. Vernez[2]. *[1]Department Environmental & Occupational Health, University of Montreal, Montreal, QC, Canada and [2]Institute for Work and Health, Lausanne, Switzerland.*

Captan is a frequently used fungicide in strawberry and orchards fields. Once orally or dermally absorbed in the human body, it is readily metabolized to a tetrahydrophthalimide (THPI) and an unstable thiosphosgene. Metabolism has been fairly well documented in in vivo studies in animals as well as in vitro studies, but human kinetic data are limited. To help better assess biomonitoring data in workers, the kinetics of ring metabolite of captan was determined in accessible biological matrices of volunteers orally and dermally exposed in controlled conditions. Five young male adults were orally exposed to 1 mg/kg of captan and, two weeks later, were dermally applied 10 mg/kg of captan on 80 cm2 of the forearm. Blood samples were withdrawn at fixed time periods over the 72 h following exposure and complete urine voids were collected over 96 h post-dosing. THPI was then quantified in these samples by HPLC-MS/MS. In plasma, peak levels of THPI were observed on average 10 and 24 h following oral and dermal exposure, respectively, and subsequent elimination phase appeared monophasic with respective mean elimination t½ of 15.7 and 24.7 h. Similar elimination t½ were calculated from excretion rate time courses (11.7 and 18.7 h, respectively). The percentage of dose recovered as THPI in urine was 3.5 and 0.02 %, respectively, after oral and dermal dosing. THPI was thus found to be rapidly formed and eliminated from the body following both oral and dermal exposure and represented a minor metabolite. However, as compared to ingestion, dermal exposure is associated with a lower absorption fraction and slower absorption rate, the latter influencing the elimination rate of THPI from the body. This kinetic study proved useful to better assess the potential use of THPI as a biomarker of exposure to captan.

PS 2279 IMPACT OF NON-LINEAR PHARMACOKINETICS AND METABOLISM OF CHLORPYRIFOS ON BIOLOGICAL RESPONSE IN THE RAT.

M. Bartels[1], M. S. Marty[1], J. A. Hotchkiss[1] and D. R. Juberg[2]. *[1]Toxicology, Dow Chemical, Midland, MI and [2]Toxicology, Dow AgroSciences, Indianapolis, IN.*

A meta-analysis was conducted on pharmacokinetic (PK) and metabolism data from numerous studies of chlorpyrifos (CPF) in the rat. This analysis shows that, in the rat, CPF undergoes a high degree of first-pass metabolism prior to reaching the systemic circulation, an effect that is more pronounced at lower doses which results in less-than dose proportional levels of CPF in the blood at lower exposures. These high first-pass metabolism data are consistent with the increased cholinesterase (ChE) inhibition seen in the blood over peripheral tissues following CPF exposure, due to pre-systemic formation of chlorpyrifos oxon (OXON) and resulting OXON-based inhibition of RBC and plasma esterases. These results are also consistent with the high percentage of ChE inhibition in blood from OXON administered at 1-10 mg/kg, with corresponding lack of inhibition in brain, indicating little to no systemic bioavailability of OXON to brain tissue. Evaluation of PK data from neonatal and adult rat studies shows that this metabolic saturation is present in both age groups, with substantially higher metabolism of CPF for doses below 1 mg/kg than those above this dose. Dietary intake affords a slower dose-rate (lower net dose/time) than bolus gavage, resulting in higher presystemic metabolism and reduced ChE inhibition effects in brain tissues. Newer kinetic data following inhalation exposure to a CPF aerosol also show comparable increases in CPF metabolism at lower exposures. In conclusion, these examples show that CPF metabolism can be affected by the dose, the exposure route, and the dose-rate in any given experiment. Lower doses and slower dose-rates can result in higher percentages of CPF metabolism, in preweanling rats as well as adult animals. Finally, these results on ChE inhibition can be expanded to evaluate potential dose-response relationships for proposed non-cholinergic effects of CPF, as the reactive OXON metabolite is expected to be the chemical moiety responsible for both types of biological effects.

PS 2280 COMPARISON OF THE EFFECT OF ASSAY CONDITIONS ON INTERPRETATION OF TIME-DEPENDENT INHIBITION OF CYTOCHROME P450 ENZYMES USING SINGLE POINT AND IC50 SHIFT ASSAYS.

K. Fox[2], R. Pearson[2], P. Butler[2], C. Dilworth[2] and K. Tsaioun[1, 2]. *[1]Apredica, Watertown, MA and [2]Cyprotex, Macclesfield, United Kingdom.*

Assessment and early detection of new chemical entities (NCEs) which inhibit cytochrome (CYP) P450 enzymes is of great importance in reducing the development cost of compounds which can cause drug-drug interactions (DDIs) and associated toxicity. In particular, irreversible time dependent inhibition (TDI) is of concern for its inhibition results in inactivated enzyme which must be re-synthesised in order to regain activity. At present there is divided opinion on experimental methods and interpretation of data, as explored by the PhRMA review (2009). The aim of this study is to evaluate different assay designs, and subsequent data analysis methodology for measuring the extent of TDI for known time-dependent inhibitors. We compared the main variations in assay design including addition of a 10x dilution step, thus pre-incubating at a higher protein concentration and increasing the substrate concentration to 5x Km. The 'shifted IC50' (IC50 determined after a 30 minute pre-incubation with human liver microsomes and

NADPH), for potent reversible inhibitors varied by the assay dilution factor, e.g. for mibefradil (irreversible and potent reversible inhibitor), shifted IC50 with 10x dilution is 0.33μM, shifted IC50 with no dilution is 0.033μM. Consequently the fold shift (ratio of IC50 30 minute pre-incubation minus NADPH/IC50 30 minute pre-incubation plus NADPH) does not change. As a result the difference between different assay conditions and the way they should be interpreted is dependent upon how the direct inhibition is accounted for. A dilution step between pre-incubation and incubation helps to reduce direct inhibition although this can skew data. By assessing the single point and IC50 shift screens we propose a reversible inhibition and TDI screening platform to cover early phase compounds, which should enable early accurate decisions to be made regarding development of those compounds which could potentially cause DDIs and associated toxicity.

PS 2281 REACTIVITY OF BRO3- EXPLAINS POOR APPARENT ABSORPTION OF LOW ORAL DOSES F344 RATS.

R. J. Bull[1], N. Kolisetty[2], Z. Guo[3], J. W. Fisher[4], J. A. Cotruvo[5] and B. S. Cummings[2]. [1]*Washington State University, Richland, WA,* [2]*University of Georgia, Athens, GA,* [3]*Center for Water Technology, Public Utility Board, Singapore, Singapore,* [4]*U.S. FDA, Jefferson, AR and* [5]*Joseph Cotruvo & Associates, LLC, Washington, DC.*

BrO3- is a by-product of ozone use to disinfect drinking water. At high doses, BrO3- has been shown to be carciongenic in F344 rats. The experiments reported here are part of an effort to characterize the pharmacokinetics of BrO3-. A first pass effect (75%) is seen at oral doses of BrO3- in the range that produce cancer. However, it has been very difficult to estimate plasma conc. of BrO3- with of doses <0.4 mg/kg. Because of its reactivity, [18O]-BrO3- was used to correct for loss of BrO3- in sample preparation. Examination of the IV clearance of BrO3- suggested an initial loss of the anion that was much more rapid than suggested by its half-life. To determine what might underlie this, the loss of BrO3- when incubated with whole blood or 20% homogenates of liver or kidney incubated at 37 deg C for 10 min. was examined. Acetonitrile was added to halt enzyme-mediated degradation. To establish a zero time value, the acetonitrile was added prior to BrO3-. It was discovered that BrO3- was very rapidly reduced to non-detectable levels when less than 66 μM was added to the zero time sample. Losses of BrO3- were recovered as Br-, establishing that it was due to reactions with reductants within these tissues. The loss of BrO3- in vitro is consistent with the apparent losses in vivo and occurs in the same concentration ranges. At greater concentrations there was evidence of a secondary degradation of BrO3- in blood that was consistent with the half-life of bromate observed when administered I.V. On the other hand, oral administration of equimolar doses of BrO3- or Br- to rats resulted in less than complete recovery of administered BrO3- as Br-, indicating that some portion of the BrO3- is involved in side reactions, most probably formation of brominated organic compounds mediated through the partial reduction product of BrO3-, HOBr. Supported by Water Research Foundation Contract 4042.

PS 2282 GENETIC VARIATION IN ISOGENIC STRAINS OF LABORATORY MICE ALTERS DISPOSITION IN KIDNEYS AND LIVERS AFTER ACUTE EXPOSURE TO [14C] BENZENE.

R. Kuester[1], G. A. Knudsen[1], M. L. Cunningham[2] and G. Sipes[1]. [1]*Department of Pharmacology, University of Arizona, Tucson, AZ and* [2]*NTP NIEHS, Montréal, QC, Canada.*

Benzene exposure is linked with aplastic anemia and hematological cancers. Exposure to low doses of benzene results in hepatic biotransformation and elimination of water soluble metabolites in the urine. Previously, we reported > 2 fold difference in several PK parameters among mouse strains in the total [14C] radioactivity for blood and bone marrow following [14C] benzene administration to a series of genetically diverse murine strains selected from the NTP-Perlegen Mouse Genome Project. Current studies investigated the disposition over time of total [14C] radioactivity in liver and kidneys of male and female mice of selected strains following a single oral dose of [14C] benzene. Mice (5 per time point) were administered [14C] benzene (0.1 mg/kg, 75 μCi/kg) and euthanized at time points between 5 min and 2 h. Kidneys and livers were excised and solubilized. Total [14C] content was quantified using liquid scintillation counting. Pharmacokinetic parameters were estimated using a one-compartment model assuming first order elimination kinetics. The AUC, CL_F and Cmax of [14C] equivalents in livers ranged from 82 to 1224 min*μmol/mg protein, 0.003-176 nmol/min* /mg protein, 0.7-2.5 nmol/mg protein, respectively. In general, the AUC values for liver were 2-3 fold greater than those of the kidneys while CL_F from the kidney exceeded that of the liver across the strains. In a few outlier strains, liver (MOLF/EiJ, KK/HiJ, A/J, C3H/HeJ, WSB/EiJ and Balb/cByJ, females and MOLF/EiJ, NOD/ShiLtJ and WSB/Ei,J males) and kidneys (A/J, females and DBA/2K males) the data could not be fit to the model either due to a failure to 'capture' the elimination phase (Cmax >120 min) or a flat elimination phase ($t1/2\beta \rightarrow \infty$). These data suggest that the disposition of benzene to the liver and kidneys is dependent upon the genetic background of a mouse. This research was supported in part by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Science Center Grant Number P3-ES-06694.

PS 2283 PYRIDAZINE REDUCES TRICHLOROACETIC ACID (TCA) BIOAVAILABILITY BY ENHANCING ITS URINARY ELIMINATION.

J. Bruckner, S. Lee, C. A. White, S. Muralidhara and R. Govindaarajan. *Pharmacology and Biomedical Sciences, University of Georgia, Athens, GA.*

TCA, a proximate mouse hepatocarcinogen, is a major metabolite of halocarbons, as well as a by-product of chlorination of water. Lee et al. (2010) found pretreatment of male Sprague-Dawley (S-D) rats with pyridazine (PZ), a potent cytochrome P4502E1 inducer, resulted in a marked decrease rather than increase in TCA bioavailability. The objective of the current study was to explore mechanisms for this unanticipated change in dosimetry. Male S-D rats received 200 mg PZ/kg ip for 3 days. Controls received saline. PZ-pretreated rats given 10 or 50 mg TCA/kg iv exhibited more rapid systemic elimination of TCA, due largely to enhanced urinary excretion. A less pronounced effect at the higher dose is suggestive of a saturable renal reabsorption process. Other PZ-pretreated and conrol rats received saline or 200 mg probenecid (PB)/kg ip 10 min before 50 mg TCA/kg iv. PB, an organic anion transporter (OAT) inhibitor, produced a modest decrease in systemic TCA clearance (CL) in controls and partially offset the marked PZ-induced increase in CL. Pilot in vitro experiments indicate P2 also inhibits H- and Na-dependent monocarboxylate transporters (MCT). These data suggest PB modestly inhibits OAT transport of TCA into urine; and PZ substantially inhibits TCA reabsorption from urine. MCT-mediated reabsorption may be a significant contributor to TCA's long half-life. Suppoted by U.S. DOE DE-FC09-02CH11109.

PS 2284 DETERMINATION OF TACROLIMUS IN RAT WHOLE BLOOD USING DRIED BLOOD SPOT ANALYSES BY LC-MS/MS.

M. Kim[1], G. Ray[1], Y. Liu[1], R. Losson[2] and G. Bricker[2]. [1]*KCAS, Shawnee, KS and* [2]*Xenometrics, Stilwell, KS.* Sponsor: D. Dandekar.

Tacrolimus (FK-506) is an immunosuppressive agent that is used to lower organ rejection following transplantation and to treat various autoimmune diseases of the skin. However, tacrolimus has significant toxicity, in particular cardiotoxicity to dogs. In the development of a novel dosage form of tacrolimus, a dried blood spot (DBS) method was desirable for toxicokinetic assessment from serial sampling from rats. DBS is an appropriate approach, since tacrolimus is highly sequestered in to red blood cells. Serial blood samples (40 μL) were removed from cannulated rats following oral gavage administration, placed onto DBS cards (Ahlstrom), and allowed to dry at ambient conditions. DBS punches where then placed into methanolic extraction solvent containing internal standard (ascomycin) and extensively vortexed using a multitube vortexor. The resulting extraction solvent was evaporated to dryness and reconstituted in mobile phase for LC-MS/MS. The samples were chromatographed using a Betasil cyano column (100x2.1 mm, 5 μ) and methanol-based mobile phase solutions. A valve switching system with a trap column (Betasil cyano, 10x2.1 mm) was used for eliminating background noise built up with injections of extracted samples. The analytes were detected by Turboion Spray API 4000 LC-MS/MS system under positive MRM mode. The quantitation ranges were 5 - 250 ng/mL. The method was suitable for PK and TK analyses with accuracy, precision and stability of the data within FDA guidelines.

PS 2285 A SENSITIVE LC-MS/MS ASSAY FOR DETERMINATION OF BENAZEPRIL AND BENAZEPRILAT IN DOG PLASMA.

C. Chung, M. Luna, Y. Liu, G. Ray and D. Dadgar. *KCAS, Shawnee, KS.* Sponsor: D. Dandekar.

Benazepril, an angiotensin-converting enzyme inhibitor, is widely used for the treatment of cardiovascular diseases. Benazepril is converted by hepatic cleavage of the ester group to the active metabolite, benazeprilat, which have both renal and hepatic elimination. In order to support an appropriate dosing form for the treat-

ment of hypertension in companion animals, a sensitive and selective LC-MS/MS method has been developed and validated for determination of benazepril and benazeprilat in dog plasma.
In this method, following a solid phase extraction procedure using Oasis WCX SPE cartridges (3cc, 60 mg) for sample cleanup, the extract was chromatographed using a Betasil phenyl column (10 X 2.1 mm, 3μ) and methanol-based mobile phase solutions. A valve switching system is used for eliminating background noise built up with injections of extracted samples. The analytes were detected by TurboIonSpray API 4000 LC-MS/MS system under positive MRM mode. The quantitation ranges were 0.1 to 20 ng/mL and 0.5 to 100 ng/mL for benazepril and benazeprilat, respectively.
Overall intra-assay accuracy [% bias] and precision [%CV] observed during validation for four levels of QC samples (six replicates each level, including the LLOQ level) in three runs were -1.5 to 0.1% and 1.8 to 10.4%, respectively, for benazepril compared to -0.5 to 0.6% and 1.7 to 10.3% for benazeprilat. Matrix effect was evaluated for benazepril and benazeprilat in six different lots of blank dog plasma at concentrations of 0.3 and 1.5 ng/mL, respectively, with variation of response [CV%] of 0.1% for both analytes. The extraction recovery, potential conversion effect from benazepril to benazeprilat, stability results, and results for incurred sample reanalysis will be presented. The validated method can be demonstrated to be reliable in drug development and GLP studies.

PS 2286 EVALUATION OF ADVERSE EFFECTS ON HUMAN LUNG FUNCTION CAUSED BY OZONE.

R. L. Prueitt[1] and J. E. Goodman[2]. [1]*Gradient, Seattle, WA and* [2]*Gradient, Cambridge, MA.*

Biological changes in response to an environmental exposure occur along a continuum that may eventually, depending on exposure dose and duration, lead to adverse effects. Recently, a framework was described that evaluates biomarkers of exposure and effect that are found on this continuum to determine whether an exposure is likely to be causal and an effect is likely to be adverse (Goodman et al., 2010). We applied this framework to estimate the ozone concentration at which short-term exposures cause respiratory effects and to characterize the degree of adversity of these effects. We evaluated human clinical studies and observational epidemiology studies that examined associations between ambient ozone levels and effects on lung function. Using the framework, we assessed causality by determining whether effects observed in these studies are statistically significantly different between exposed and non-exposed subjects, isolated or independent, secondary, observed because of study limitations, or exposure related but unrelated to the apical effect. We assessed the degree of adversity of the observed effects by determining whether they are adaptive or compensatory, transient, reversible, early precursors of an apical effect, of low severity, or do not result in functional impairment. Our analysis indicates that the available evidence supports a short-term exposure threshold of 70 ppb for effects on lung function, although these effects do not meet the criteria for adversity as specified in the framework. Below this dose, effects are isolated or independent and not statistically different in exposed subjects in clinical studies, and study limitations affect interpretation of results from observational studies. The available evidence indicates that adverse effects are observed at ozone exposures of at least 87 ppb, and these effects are reversible and of low severity. We conclude that the available evidence supports a short-term exposure threshold of 70 ppb ozone for effects on lung function, with adverse effects occurring at ozone concentrations of at least 87 ppb.

PS 2287 MODELING NF-κB REDOX SENSITIVITY: APPLICATIONS TO OZONE-INDUCED LUNG INFLAMMATION.

D. J. Miller and P. D. White. *U.S. EPA, Washington, DC.* Sponsor: S. Vulimiri.

The NF-κB intracellular signal transduction pathway plays a well-established role in inflammatory signaling, including transcriptional control of a variety of cytokines that are critical to inflammatory response to ozone within lung airway epithelial cells. Less understood are the mechanisms involved in NF-κB pathway activation in the presence of oxidative stress, despite the absence of canonical pathway receptor stimuli. We have constructed a redox-sensitive pathway model that is based upon multiple biological components described in recent literature. This includes a central role for oxidative inhibition of phosphatases, leading to a buildup of upstream kinase activity and subsequent downstream signaling of NF-κB. Computational modeling of this pathway elucidates these testable mechanisms of activation, and systematic perturbation of the model informs our understanding of sensitive subpopulations by identifying pathway components that are most critical to regulation of pathway response. As part of a larger multidisciplinary effort to apply such next-generation approaches to risk assessment, we describe this model of the NF-κB signaling pathway, and demonstrate both (1) a potential mechanism for pathway activation via intracellular reactive oxygen intermediates generated by ozone and (2) applications of the model to informing human risk.

PS 2288 COMBINED CFD/PBPK MODELS FOR DETERMINING SITE-SPECIFIC UPTAKE AND TISSUE CONCENTRATIONS OF REACTIVE GASES IN THE RESPIRATORY TRACT OF RATS AND HUMANS.

R. A. Corley[1], S. Kabilan[1], J. E. Carson[1], R. Jacob[1], K. R. Minard[1], R. W. Glenny[2], S. Pipavath[2], M. Fanucchi[3] and D. R. Einstein[1]. [1]*Pacific Northwest National Laboratory, Richland, WA,* [2]*University of Washington, Seattle, WA and* [3]*University of Alabama at Birmingham, Birmingham, AL.*

3D computational fluid dynamic (CFD) airflow models have been developed for the full respiratory system of rats and humans. The CFD models include the external nares and mouth to provide more realistic airway inlets for nasal vs. oral breathing. Site-specific airflow patterns are heavily influenced by species-specific 3D anatomy and breathing patterns. Since potentially significant human exposures to reactive gases occurs via cigarette smoking, we incorporated a two-compartment PBPK model for acetaldehyde as airway boundary conditions for the CFD model to demonstrate the impact of using full respiratory airflow models in comparative target tissue dosimetry. The airway PBPK models were compartmentalized according to species-specific cell type (nose) or anatomic region (rest of respiratory tract). Physiological constants for the two compartments representing mucus and surface epithelium (compartment 1) and submucosa with blood perfusion (compartment 2) were taken from the literature or measured directly. Chemical-specific constants were taken from the PBPK model of Teeguarden et al. Inhal. Toxicol. 20 (2008) 375-390. Site-specific flux rates, Cmax's, AUC's and Lifetime average Daily Dose (LADD) levels for each compartment and region were determined at the NOAEL for subchronic nasal olfactory degeneration in the rat (50 ppm) and at an average yield of acetaldehyde in cigarette smoke for humans. Although the concentrations of acetaldehyde can achieve very high initial concentrations (>1000 ppm) in the mouth, the transient nature of a bolus, puff inhalation results in 13- to 50-fold lower LADD's in human tissues than the rat olfactory region depending upon the number of cigarettes smoked/day, with the larynx in humans being the region of greatest tissue concentration following oral inhalation. Funded by NHLBI R01 HL073598 and RJR Project 56296.

PS 2289 MODELING VAPOR UPTAKE AND TISSUE DISPOSITION IN HUMAN LUNGS.

M. Singal[1], B. Asgharian[2], O. T. Price[2], J. S. Schroeter[3] and J. S. Kimbell[4]. [1]*Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ,* [2]*Health Effects & Medical Response, Applied Research Associates, Raleigh, NC,* [3]*Division of Computational Biology, The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [4]*Department of Otolaryngology/Head and Neck Surgery, University of North Carolina, Chapel Hill, NC.*

Estimates of the uptake of inhaled vapors in the lung and transport to other tissues and organs of the body are needed to assess body burden and biological response. Vapor transport through the lung is usually modeled as a steady flow process through a representative cylindrical tube or simply an input parameter to a pharmacokinetic (PK) model. When the target site in toxicology studies is the lung and improved PK predictions are desired, a mechanistic model is needed that can predict regional lung vapor uptake and tissue concentration using a realistic lung geometry and ventilation pattern. A vapor uptake model in the airways and adjacent tissues was developed based on the momentum and mass conservation of air and inhaled material through the airways. Mass transfer coefficients for unsteady breathing during inhalation, pause, and exhalation were developed to uncouple transport equations in the air and tissue phases. Vapor transport in the tissue occurred by diffusion while the absorbed vapor was eliminated by linear and saturable pathways. The model was used to study the fate of vapors of different solubilities and reactivities in the tissue. Highly soluble vapors such as formaldehyde were found to reach only the first few airway generations of the lung while less soluble vapors such as acetaldehyde penetrated deep into the lung with a significant accumulation in the lung tissue at the end of a breathing cycle. A lung-tissue vapor uptake model coupled with a PK model alleviates, or reduces the need for parameter optimization and enables capturing physical and biological mechanisms of action to study potential health effects of exposure to vapors.

PS 2290 DOSIMETRY OF SOLUBLE, REACTIVE VAPORS IN THE UPPER RESPIRATORY TRACT OF THE SPRAGUE-DAWLEY RAT USING A COMPUTATIONAL FLUID DYNAMICS MODEL.

J. Schroeter[1], J. S. Kimbell[2], B. Asgharian[3] and M. Singal[4]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC,* [2]*Otolaryngology/Head and Neck Surgery, University of North Carolina, Chapel Hill, NC,* [3]*Applied Research Associates, Inc., Raleigh, NC and* [4]*Research Institute for Fragrance Materials, Inc., Woodcliff Lake, NJ.*

Regional estimates of respiratory dosimetry are needed to study potential health effects from exposure to inhaled soluble and reactive vapors due to their large concentration gradients throughout the respiratory tract. A three-dimensional computational fluid dynamics (CFD) model of the upper respiratory tract (including nasal passages, nasopharyngeal duct, larynx, and anterior trachea) of a Sprague-Dawley rat was used to predict uptake of formaldehyde, acrolein, and acetaldehyde. Solubility, diffusivity, and reactivity parameters were obtained from the published literature and were used in the boundary condition to calculate compound-specific wall mass flux. Excellent agreement was observed between nasal uptake predictions using the CFD model and experimental data from previous in vivo studies. Predicted nasal uptake fractions were 94%, 67%, and 50% for formaldehyde, acrolein, and acetaldehyde, respectively, at an inhalation exposure concentration of 1 mg/m^3 and an inspiratory flow rate of 737 ml/min, equal to twice the estimated minute volume of the rat. Nasal uptake of formaldehyde was independent of exposure concentration. Nasal uptake of acrolein and acetaldehyde displayed nonlinear behavior with increased uptake as exposure concentration decreased. Wall flux of formaldehyde was highest in the anterior region of the nose due to its high solubility and exhibited a sharp concentration gradient. Wall flux of acrolein and acetaldehyde exhibited less of a gradient than formaldehyde, but still displayed highly nonuniform uptake patterns throughout the nasal passages. Nasal dosimetry calculations using CFD models can be used for interspecies extrapolation of localized dose to predict health risks in humans.

PS 2291 MODELS FOR EVALUATION OF THERAPEUTIC INTERVENTION OF INTERNALLY DEPOSITED AMERICIUM.

R. A. Guilmette[1], W. M. Weber[1], M. Doyle-Eisele[1], G. Miller[2], L. Bertelli[2] and J. D. McDonald[1]. [1]*Lovelace Respiratory Research Institute, Albuquerque, NM and* [2]*Los Alamos National Laboratories, Los Alamos, NM.*

To be able to quantitatively evaluate the efficacy of new decorporating agents, several steps are required; 1) development of one or more animal models that provide data on the unperturbed biokinetics of the radionuclide; 2) testing the efficacy of the chelating agent at different doses and treatment schedules; and 3) use these data to parameterize biokinetic and dosimetric models that describe the behavior of treated or untreated animals. Models of internal exposure to Am241 have been established. An initial determination of the efficacy of the chelating agent diethylenetriaminepentaacetic acid (DTPA) has been evaluated using the methodology described above. Biokinetic results from rats given Am241 intravenously in the absence of DTPA showed that approximately 2.7% of the injected dose (ID) was removed in feces 2 days after administration. However, when DTPA was administered intravenously 1 hour after Am241, 39.1% ID was removed from feces 2 days after Am241. The biokinetics of the Am241 dose were further evaluated with a custom multicompartmental computer model. This model, RatDose, provided total radiological dose as a function of time and tissue. These data, and the novel model, can help to support approval of drugs according to current US Food and Drug Administration guidance under the conditions of the Animal Rule.
Work performed under contract No. HHSN266200500043C with the University of Maryland and the National Institute of Allergy and Infectious Disease.

PS 2292 MODELING FIBER INHALABILITY AND DEPOSITION IN THE HUMAN AND RAT RESPIRATORY TRACT.

B. Asgharian[1], O. T. Price[1], S. H. Gavett[2] and A. M. Jarabek[2]. [1]*Health Effects & Medical Response, Applied Research Associates, Raleigh, NC and* [2]*U.S. EPA, Research Triangle Park, NC.*

Exposure to airborne asbestos fibers may lead to various respiratory health diseases such as asbestosis, mesothelioma, and lung cancer. To study the health effects of inhaled asbestos fibers and to assess risk from environmental or occupational exposures, exposure-dose-response relationship must be characterized. Dosimetry models aid that characterization by predicting internal dose across species for different dose metrics relevant to the mode of action. The first step in fiber dosimetry model development is to address inhalability. Our model for inhalability was developed using equivalent fiber impaction diameters derived and substituted in expressions for spherical particle diameters to determine fiber inhalability and nasal losses. A deposition model component describes the extra-thoracic deposition to determine the fraction of inhaled fibers that reach the lower respiratory tract (LRT), and a LRT deposition model predicts the deposited dose of fibers in different regions of the LRT. Three components comprise the deposition model: airway geometry, airway ventilation, and a mathematical formulation of transport and deposition. The first 2 components are common between particles and fibers, and the multi-path particle dosimetry (MPPD) model [MPPD version 2.0; ARA, NC] was used. However, fiber transport and deposition in the airways are different from those for particles. Thus, new models of deposition efficiencies of fibers by mechanisms of sedimentation, impaction, interception, and diffusion were derived for the head and LRT airways by replacing particle hydrodynamic drag forces in transport equations with those for fibers. Predicted internal fiber doses in different regions versus exposure illustrate the potential impact on response analyses. Fiber deposition depends strongly on both fiber length and diameter dimensions, emphasizing the need for bivariate fiber distribution characterization of air exposures. (These views are the authors and do not represent US EPA policy).

PS 2293 TRANSLATION OF INHALED FIBER DOSE ACROSS SPECIES AND DIFFERENT EXPERIMENTAL DESIGNS: FIBER EQUIVALENT DIAMETER.

O. Price[1], B. Asgharian[1], S. H. Gavett[2] and A. M. Jarabek[2]. [1]*Applied Research Associates, Inc., Raleigh, NC and* [2]*U.S. EPA, Research Triangle Park, NC.*

Inhaled fibers of all lengths have been shown to induce pathological responses, but different sizes are respirable in different species. To accurately assess the health effects observed in toxicological or epidemiological studies, it is necessary to determine respirable fiber doses for in-vivo and in-vitro studies, and to be able to translate that dose to a human equivalent air exposure. The preparation of an appropriate respirable fiber test sample is a critical and challenging first step of toxicological studies. Traditionally, approaches to size selection have been based on particle aerodynamic diameter and the use of centrifuges or cyclones which separate by coriolis or centrifugal forces. Unlike particles, fibers cannot be simply described by a single diameter since both length and width determine their aerodynamic properties, so such separation techniques cannot be extended to fibers without proper modifications. Further, sedimentation is the basis for settling velocity whereas examination of fiber transport mechanisms shows that the ability of fibers to enter the respiratory tract is determined by inertial properties. Thus, an equivalent fiber diameter for impaction needs to be defined and used as the basis for size selection. We first calculate a particle impaction diameter to show the difference in respirable estimates based on mechanism. We then show the fiber equivalent impaction diameter, assuming a random orientation as a function of fiber diameter and aspect ratio. We further illustrate the influence of using a bivariate distribution function rather than the aspect ratio to characterize the fiber size based on a range of fiber parameters for the joint correlation between length and diameter distributions. The impact on dose estimates for various toxicological studies is depicted, and correction factors derived to compensate for these considerations across experimental designs. (These views are the authors and do not represent US EPA policy.)

PS 2294 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR THE DISPOSITION OF PROPYL SERIES COMPOUNDS (PROPYL ACETATE, PROPANOL, PROPIONALDEHYDE, AND PROPIONIC ACID) IN RATS.

L. M. Sweeney[1, 2] and T. S. Poet[3]. [1]*Naval Medical Research Unit-Dayton, Wright-Patterson Air Force Base, Wright-Patterson Air Force Base, OH,* [2]*Toxicology Excellence for Risk Assessment, Cincinnati, OH and* [3]*Battelle Pacific Northwest National Laboratory, Richand, WA.*

A validated physiologically based pharmacokinetic (PBPK) model for disposition of the propyl series compounds (propyl acetate, propanol, propionaldehyde, and propionic acid) in the rat has been developed. This effort built upon earlier human PBPK modeling work for these compounds, a preliminary rat PBPK model for these compounds, new in vivo data from rat studies, and insights derived from the modeling of structurally-related compounds. Specifically, the newer data indicated that the previous rat model overpredicted urinary excretion of propanol. A revised model consistent with the newer data has been developed. The structure and parameter values of this model are generally consistent with previously developed models for structurally similar chemicals; aldehydes exhibit the greatest differences in parameter estimates. The level of agreement between the propyl series model predictions and the experimental data is, on average, within a factor of about 3 for most of the available in vivo data sets. Together, the rat and human PBPK models

for the propyl series can be used for extrapolation of results of rat toxicology studies (e.g., no-observed adverse effect levels) to human-equivalent values for exposure to propyl acetate or propanol.

PS 2295 USING MARGINS OF EXPOSURE AS A SEGREGATION TOOL FOR RISK ASSESSMENT OF TOBACCO SMOKE TOXICANTS.

S. Fiebelkorn, F. H. Cunningham and C. Meredith. *Group R&D, British American Tobacco, Southampton, United Kingdom.*

Identification of tobacco smoke toxicants has received significant attention during recent years. Key examples include establishment of the World Health Organisation (WHO) Study Group on Tobacco Product Regulation (TobReg) and most recently, in 2009, the Family Smoking Prevention and Tobacco Control Act. There is a clear focus to develop lists of tobacco toxicants which may guide proposal of limits, recommendations for lowering and/or routine monitoring. We suggest development of a quantitative risk assessment paradigm, using multiple assessment techniques to estimate the contribution of individual toxicants to smoking-related diseases, with the aim of establishing priorities for risk reduction research. As an initial model, we propose adoption of the Margins of Exposure (MOE) model, following European Food Safety Authority (EFSA) guidelines. An MOE is a ratio between a benchmark dose (derived from existing toxicological dose-response data) and estimated human exposure. Compounds with computed MOEs >10,000 are considered to be "low priority for risk management actions". Our approach is to calculate MOE values for individual tobacco smoke toxicants from a range of published toxicity studies and from published data on yields from machine smoking to determine consistency within available data sets. Computed MOEs enable segregation of toxicants into high and low priority groupings for risk reduction research depending on their relationship to the critical MOE value of 10,000. For example, following this procedure, carcinogenicity data sets for 1,3-Butadiene yield MOEs all below 10,000 whereas MOEs for vinyl chloride are all above 10,000. Using non-cancer end-points for cresol(s) yields MOEs that are all above 10,000. We propose that estimation of MOEs for smoke toxicants is a useful first step towards prioritisation of toxicants for risk reduction research. Future developments will invoke more complex, but physiologically more relevant paradigms, such as physiologically-based pharmacokinetic (PBPK) modelling to ascertain the importance of a toxicant to smoking-related diseases.

PS 2296 USE OF CONTROL BANDING AND SENSORY IRRITATION (RD_{50}) DATA TO ASSESS OCCUPATIONAL EXPOSURE VALUES FOR N-PROPYL, N-BUTYL, AND N-PENTYL PROPIONATE.

S. M. Krieger[1], D. R. Geter[1], T. J. Cawley[1], M. Osterloh-Quiroz[2] and J. A. Hotchkiss[1]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Dow Europe GmbH, Horgen, Switzerland.*

N-propyl, n-butyl, and n-pentyl propionate are colorless liquid chemical intermediates used in the production of pharmaceuticals, anti-fungal agents, agrochemicals, plastics, plasticizers, rubber chemicals, dyes, artificial flavors, and synthetic perfumes. In addition, they are used as solvents in low VOC coating formulations. Inhalation is the primary route of potential occupational exposure to these materials. No occupational threshold limit values (TLV) are currently established for this solvent family. Atrophy, degeneration and necrosis of the olfactory epithelium are the most sensitive repeat inhalation observations of the above mentioned propionates. Inhalation No-Observed-Effect Concentrations (NOEC) range from 50-500 and 50-750 ppm for subacute and subchronic toxicity, respectively; 500-2000 ppm for reproductive and developmental toxicity, and 500-3200 ppm for neurotoxicity. A control banding (matrix) approach may be used to identify a range of permissible occupational exposure concentrations. Based on the available repeat inhalation-exposure data, TLVs in the range of 1-10 ppm for nasal toxicity and 10-100 ppm for systemic toxicity were derived. Perceived irritation of the nose, throat and eyes is recognized as a critical effect and often used to derive TLVs. The TLV for many irritant chemicals falls within a range 0.01 - 0.1 RD_{50}, with 0.03 often used as the appropriate value (RD_{50} = concentration causing a 50% reduction in baseline respiration rate). We experimentally established RD_{50} values of 535 and 668 ppm for n-propyl and n-butyl propionate, respectively (ASTM Method E 981-04). Calculated TLVs based on 0.03 times the RD_{50} yielded values of 16.1 and 20.0 ppm for n-propyl- and n-butyl propionate, respectively. These values are in agreement with the repeat inhalation derived TLVs and support the use of RD_{50} in calculating these data.

PS 2297 ASSESSING NON-CANCER HEALTH RISK FROM INHALED PCBS.

G. M. Lehmann[1] and J. Schaum[2]. [1]*ORD, U.S. EPA, Research Triangle Park, NC and* [2]*ORD, U.S. EPA, Washington, DC.*

Exposure to polychlorinated biphenyls (PCBs) has been associated with adverse health effects in humans, including hepatotoxicity, altered thyroid hormone homeostasis, immunotoxicity, reproductive effects, and developmental neurobehavioral toxicity. Contaminated food consumption has historically been the major contributor to PCB body burden, but contamination of indoor air may also contribute in some exposure scenarios. Although chronic oral reference values have been derived for PCBs, derivation of inhalation values is challenging due to a profound lack of data. Dosimetric adjustments are sometimes applied to use oral reference values as a basis for derivation of inhalation reference values. Such route-to-route extrapolation introduces uncertainty into human health risk analysis, as it requires certain basic assumptions in order to establish similarity between the oral and inhalation routes. With PCBs, further uncertainty results from differences between oral and inhalation exposures unique to this chemical class. These differences occur because PCBs are chemical mixtures made up of a variety of individual congeners. The degree of congener chlorination in a PCB mixture is an important determinant of the mixture's physicochemical properties and toxicity. Volatile congeners tend to be less chlorinated, less persistent, and less toxic than those found in food. Chronic oral reference values are currently available for two PCB mixtures: Aroclors 1016 and 1254. Aroclor 1016 contains lower-chlorinated PCB congeners than Aroclor 1254. Because inhalation exposure favors lower-chlorinated PCB congeners, the Aroclor 1016 reference value may be more representative than the Aroclor 1254 value for assessment of inhalation exposure risk. However, uncertainties remain regarding the relative toxicities of these PCB mixtures and the application of their oral reference values to inhalation exposure risk. This presentation will explore these uncertainties and identify critical areas of research needed to improve risk estimation for inhaled PCBs. The views expressed here do not reflect the views or policies of the U.S. EPA.

PS 2298 STRUCTURE-ACTIVITY MODELS FOR CHEMICAL INHALATION HEALTH GUIDANCE VALUES.

C. J. Collar[1], T. Miller[2], R. M. Garrett[2] and E. Demchuk[1]. [1]*Division of Toxicology and Environmental Medicine, ATSDR/CDC, Atlanta, GA and* [2]*Department of Defense, Washington, DC.* Sponsor: B. Fowler.

Acute Exposure Guideline Levels (AEGLs) are thoroughly examined data for airborne concentrations of hazardous chemicals. These chemical concentrations are defined as the human threshold limits at five exposure periods ranging from ten minutes to eight hours. Health effects are categorized by the National Academy of Sciences (NAS) as non-disabling (AEGL-1), disabling (AEGL-2), and lethal (AEGL-3). However, AEGLs are finalized for less than sixty compounds, and less than two-hundred are considered interim; far less than the number of potential airborne hazards to the human population. We employed the AEGLs data at the eight hour exposure period, to construct quantitative structure-activity relationship (QSAR) models capable of predicting provisional health guidance values (HGVs) for airborne chemical hazards that have no guidance assigned by the NAS. These models are statistically significant with R^2 and Q^2 values greater than 0.70 and 0.50, respectively. Since QSAR modeling is driven by uniformity and quantity of underlying data, point-of-departure and endpoint grouping factors can be employed to gain insight into how the model is formulating HGV estimates and what can be done to increase the estimation performance. Hence, we have begun this process by assessing the point-of-departure information. The AEGLs datasets were split into subsets of human and rat data and new models were built. With statistical significance comparable or better than parent models, these species-specific models were individually analyzed and also employed to validate each other; the compounds of the first model were used as a testing dataset for the second model and *vice versa*. In the future we plan to: (1) employ additional endpoint grouping factors, (2) model the remaining four time periods, and (3) incorporate other HGVs into the models.

PS 2299 THORACIC DAMPING AND THE RELATIONSHIP BETWEEN PENH OF THE THORACIC AIR-FLOW (I_T) AND TIDAL MIDEXPIRATORY FLOW (EF_{50}).

D. G. Frazer, J. S. Reynolds, W. T. Goldsmith, W. G. McKinney, M. C. Jackson and A. A. Afshari. *HELD, CDC / NIOSH, Morgantown, WV.* Sponsor: A. Hubbs.

The thoracic air-flow pattern can be modeled as a response to the action of the respiratory muscles. That response can be viewed as being either under-damped, when I_T is initially higher then decays toward the end of exhalation, or damped when I_T is initially lower and then rises toward the end of exhalation. Both Penh(I_T) and the

EF_{50} tend to decrease as I_T damping increases. Previously, it has been reported that the EF_{50} is reduced when pulmonary conductance, $1/R_L$, decreases (Glaab *et al.*, Am J Physiol. Lung Cell Mol. Physiol., 2001). In addition, it has been shown that exposure to an inhaled intervention causes an increase in Penh(I_{BOX}) where I_{BOX} represents airflow into and out of a whole body plethysmograph. The increase in Penh(I_{BOX}) results in the Penh(I_T) versus $R_{aw}C_G$ relationship moving upward and to the right (Frazer *et al.*, J. Tox. and Environ. Health, 2010). Several authors have provided evidence that not all increases in Penh(I_{BOX}) are caused by an increase in $R_{aw}C_G$ (Bates *et al.*, Am. J. of Respir. Cell and Mol. Biology, 2004). It can be shown theoretically, however, that an increase in damping of the I_T waveform following an intervention, in cases where Penh(I_{BOX}) increases, results in a reduction in Penh(I_T) and indicates an increase in $R_{aw}C_G$ (S_{RAW}). A decrease in the EF_{50} can also be shown to be related to a decrease in I_T damping.

PS 2300 FRONT-LINE CELLS IN LUNG DISEASE: SUB-CELLULAR LOCALIZATION OF PROTEINS WHOSE STATES ARE DEPENDENT ON THAT OF CDKN2A.

G. Acquaah-Mensah. *Massachusetts College of Pharmacy & Health Sciences, Worcester, MA.*

Millions of people worldwide suffer from diseases of the lung including cancer, asthma, and chronic obstructive pulmonary disease (COPD). The cyclin-dependent kinase inhibitor 2A (CDKN2A) gene has at least two known products: p16INK4A and ARF. Both act as tumor suppressors, are expressed less in a number of neoplasms including lung cancer, and could be involved in other lung diseases. Epithelial cells of the lung are front-line targets for inhaled substances. Established statistical dependencies between the expressions of pairs of genes could be indicative of regulatory relationships; they could also indicate shared participation in key processes, or shared sub-cellular localization. A central regulatory role for CDKN2A in these cells was hypothesized. The purpose of this study was to investigate the extent to which products of genes whose expressions are statistically tied to that of the CDKN2A gene co-localize with products of CDKN2A in lung epithelial cells. A compendium of microarray data was created based on lung epithelium arrays GDS534, GDS999, GDS2604, and GDS2486 obtained from the Gene Expression Omnibus repository. Using the Context Likelihood of Relatedness algorithm, statistical dependencies between the expressions of genes including those involved in apoptosis, the response to oxidative stress, and the inflammatory response were established. For each gene statistically dependent on CDKN2A, the primary amino acid sequence of the corresponding protein was used as a basis for predicting sub-cellular localization. Specifically, a matrix capturing composition, transitions, and distribution of amino acid side-chain physicochemical properties such as hydrophobicity, normalized van der Waals volume, polarity, polarizability and electric charge was used. On this basis, a support vector machine predicted a vast majority of the 315 proteins connected to CDKN2A are localized in the nucleus (43%), the plasma membrane (23%), and the cytosol (19%). This is consistent with literature and has implications for toxicant interactions in these front line lung cells.

PS 2301 HOW MUCH FORMALDEHYDE EXISTS IN EXHALED BREATH?

J. E. Whalan, D. DeVoney and S. V. Vulimiri. *U.S. EPA, Washington, DC.*

An evaluation of formaldehyde risk may be informed by endogenous levels in exhaled breath. Therefore, a recently released draft Integrated Risk Information System (IRIS) assessment has systematically reviewed the literature regarding exhaled formaldehyde in humans (http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=223614). Using proton transfer reaction mass spectroscopy (PTR-MS), several studies have reported formaldehyde in the exhaled breath of healthy volunteers ranging from below the limit of detection (3-5 ppb) to 12 ppb. An artifact of the PTR-MS method is that a portion of the mass of exhaled methanol and ethanol is misidentified as formaldehyde, which results in an overstated formaldehyde concentration. None of these studies adjusted for this artifact. Because all subjects inhaled room air containing formaldehyde, it is difficult to ascertain whether the exhaled formaldehyde was of exogenous or endogenous (systemic) origin. Thus, it was impossible to accurately quantify a formaldehyde concentration in exhaled breath that can be attributed to endogenous sources. In a recent study, Riess et al. (2010) compared an acetylacetone (acac) method to the PTR-MS method for measuring formaldehyde in the exhaled breath of healthy subjects inhaling formaldehyde-free air. Exhaled formaldehyde concentrations for all subjects were <0.5 ppb (limit of detection) versus 0.7-2.3 ppb using the acac and PTR-MS methods, respectively. This study indicates that the PTR-MS method may not be reliable for measuring formaldehyde in exhaled breath and suggests that formaldehyde concentrations in exhaled breath may be lower than previously reported. An analysis of the available data will be presented.

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PS 2302 DNA DAMAGE AND OXIDATIVE STRESS IN RATS INTRATRACHEALLY EXPOSED TO LOW-LEVEL COMBINED COBALT AND CHROMIUM.

N. Yoshioka[1], K. Hosoda[1], H. Nakashima[2], T. Michikawa[1], M. Nakano[1] and K. Omae[1]. [1]*Department of Preventive Medicine and Public Health, Keio University School of Medicine, Tokyo, Japan and* [2]*Department of Preventive Medicine and Public Health, National Defense Medical College, Tokorozawa, Saitama, Japan.*

Human are exposed daily to low level of environmental and occupational metals. Therefore, results of the toxicity tests which conducted on single-chemical at high level exposure are difficult to extrapolate to the risk assessment of the chemical mixtures in these environments. In this study, we focused on cobalt and chromium which are recognized as carcinogens with similar mode of action, and evaluated whether the DNA damage in rat lung *in vivo* exposed to mixture of two metals at low level was additive, synergistic or antagonistic. F344 Rats were intratracheally exposed to single or binary metals in five dose groups each once per week. After four weeks exposure, we measured DNA damage in lung and 8-hydoxy-2'-deoxyguanosine (8-OHdG) in plasma. DNA damage induced by metals in lung was evaluated by a comet assay. We confirmed significant dose-related increase in the DNA damage score (%DNA in tail) after single and binary metals exposure. 50% effective dose (ED50) of DNA damage was calculated by probit analysis. In this study, increase in %DNA in tail more than 3% compared to non treated sample was regard as positive effect. ED50 of Co, Cr and Co-Cr were converted from mg/kg(body wt)/week to mg/m^3 using the equation by Lewis et al., and were $0.139 mg/m^3$ (95%CI 0.094-0.205), $0.288 mg/m^3$ (0.2-0.423) and Co:0.072 mg/m^3 (0.051-0.098)-Cr:0.072 mg/m^3 (0.051-0.098) respectively. These data were subjected to isobolographic analysis to test interaction between cobalt and chromium at low level. Binary mixture elicited additive DNA damage. Likewise, 8-OHdG was also additive effect at low level binary exposure. These results support that mixed chemicals which share similar mode of action are dose additive. This may have implications for risk assessment of exposure to metals induced oxidative stress.

PS 2303 MODELING MIXTURES OF ENVIRONMENTAL ESTROGENS DETECTED IN U.S. SURFACE WATERS WITH AN *IN VITRO* ESTROGEN MEDIATED TRANSCRIPTIONAL ACTIVATION ASSAY (T47D-KBLUC).

D. S. Bermudez[1, 2], L. Gray[2] and V. S. Wilson[2]. [1]*Molecular Biomedical Sciences, NCSU, Raleigh, NC and* [2]*Reproductive Toxicology Branch, U.S. EPA, Research Triangle Park, NC.*

There is growing concern of exposure to fish, wildlife, and humans to water sources contaminated with estrogens and the potential impact on reproductive health. Environmental estrogens can come from various sources including concentrated animal feedlot operations (CAFO), municipal waste, agricultural and industrial effluents. Furthermore, U.S. EPA's drinking water contaminant candidate list 3 (CCL3) includes several estrogenic compounds. While these contaminants are currently not subject to any proposed or promulgated national primary drinking water regulations, they are known or anticipated to occur in public water systems and may require future regulation under the Safe Drinking Water Act. Using an in vitro transcriptional activation assay, this study evaluated estrogens from CCL3 both individually and as a seven estrogen mixture (fixed ray design). In addition, mixtures that mirror primary estrogens found in swine, poultry and dairy CAFO effluent (fixed ray design), and a ternary mixture (4x4x4 factorial design) of estrogens found in hormone replacement therapy and/or oral contraceptives were tested. Mixtures were evaluated for additivity using both the concentration addition model (CA) and estrogen equivalence model (EEQ). Log EC50 and Hillslope values were as follows: Estrone, -11.92 M, 1.283; Estradiol-17α, -9.61 M, 1.486; Estradiol-17β, -11.77 M, 1.494; Estriol, -11.14 M, 1.074; Ethinyl Estradiol-17α,12.63, 1.562; Mestranol, -11.08 M, 0.809; Equilin, -11.48 M, 0.946. In all cases, the CA and EEQ models predicted the observed responses accurately. There was no evidence that any of the mixtures acted synergistically or in an antagonistic manner. Results indicate both additive models are appropriate for modeling estrogen mixtures. This study augments the understanding of estrogen mixture interactions. Abstract does not necessarily reflect EPA policy. NIEHS/EPA Interagency RW75-92285501-1; NCSU/EPA Cooperative Training Agreement CT833235-01-0.

PS 2304 *IN VITRO* EXPOSURE AND EVALUATION OF MILITARY FUELS AND BIOFUELS.

K. L. Mumy[1], T. L. Doyle[1], M. R. Okolica[1], R. P. Adkins[1] and G. T. Eldridge[2]. [1]*Naval Medical Research Unit - Dayton, Wright-Patterson AFB, OH and* [2]*Naval Air Warfare Center Aircraft Division, Patuxent River, MD.*

Widespread use of jet and marine fuels continues to make exposure risks a large concern for military personnel. Recent advances in alternative fuels have provided many new fuels for potential military use in the form of biofuels produced from fats, oils, plant materials, etc. Despite being biologically-based, biofuels must still be evaluated for their toxicity and biological effects. Two biofuels of interest to the Navy are camelina and algal-based fuels to be blended with conventional fuels. Initial toxicological evaluation of a camelina-based jet biofuel and algal-based marine biodiesel indicates that these biofuels are non-mutagenic, as determined by the bacterial reverse mutation assay, and less cytotoxic when compared to the standard fuels, jet propulsion (JP)-5 and diesel fuel marine F76. An *in vitro* exposure to fuel vapors alone revealed <50% of human lung epithelial (NL-20) cells were viable after 24 h exposure to JP-5 vapor, while >90% remained viable following exposure to camelina-based fuel vapor. However, the blended fuel vapor yielded viabilities similar to JP-5 alone. Similarly, <70% of NL-20 cells were viable following exposure to F76 vapor, whereas >95% were viable after algal-based biodiesel vapor exposure, and exposure to the blended fuel vapor yielded viabilities comparable to F76 alone. Compounds present in the vapor phase of each of the fuels were identified by solid phase microextraction with gas chromatography/mass spectrometry. This method provides a screening tool to more appropriately mimic inhalation exposures to fuel vapors using an *in vitro* system and identify components of complex mixtures that may act directly at the cellular level to aid in identifying modes of action. Biologically-based fuels provide a promising avenue in the development of alternative fuels and these *in vitro* methodologies provide rapid and cost-effective means of initially screening their toxicological effects in comparison to conventional fuels.

PS 2305 DOSE-RESPONSE EVALUATION OF NON-ADDITIVE HEPATIC EFFECTS OF PCB153 AND TCDD IN MICE.

A. K. Kopec[1, 2], M. L. D'Souza[1, 2], B. D. Mets[1], L. D. Burgoon[1, 2], J. R. Harkema[2, 3], C. Tashiro[4], D. Potter[4], B. Sharratt[4], S. E. Reese[5], K. J. Archer[5] and T. R. Zacharewski[1, 2]. [1]*Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI,* [2]*Center for Integrative Toxicology, Michigan State University, East Lansing, MI,* [3]*Pathobiology & Diagnostic Investigations, Michigan State University, East Lansing, MI,* [4]*Wellington Laboratories, Inc., Guelph, ON, Canada and* [5]*Biostatistics, Virginia Commonwealth University, Richmond, VA.*

Interactions between environmental contaminants, such as dioxins and PCBs, can lead to non-additive effects that may influence the risk assessment of a mixture. A comprehensive dose-response evaluation of hepatic effects elicited by TCDD, non-dioxin-like PCB153 and their mixture was performed in immature, ovariectomized C57BL/6 mice. Mice were orally gavaged once with TCDD (0.3, 1, 3, 6, 10, 15, 30, 45 μg/kg), PCB153 (3, 10, 30, 60, 100, 150, 300, 450 mg/kg), a mixture (MIX: 0.3+3, 1+10, 3+30, 6+60, 10+100, 15+150, 30+300, 45+450 μg/kg TCDD + mg/kg PCB153, respectively) or sesame oil vehicle, and sacrificed 24 h post dose. TCDD and MIX induced significant increases in relative liver weights (RLW) in all but the lowest dose groups, while PCB153 significantly induced RLW only at 100 and 300 mg/kg. Complementary histopathology and gas chromatography/mass spectrometry identified significant increases in lipid levels in the MIX groups compared to either vehicle, TCDD or PCB153 alone. Hepatic PCB153 levels were also significantly increased following TCDD co-exposure. Nonlinear logistic modeling with quasi-likelihood estimates identified significant dose-dependent, non-additive expression of Pla2g12a, Serpinb6a, Nqo1, Srxn1 and Dysf in MIX groups compared to TCDD or PCB153. Transcription factor binding site analysis identified putative dioxin, constitutive androstane and pregnane X receptor binding motifs in the promoter region of non-additive target genes suggesting receptor cross-talk. Collectively, the dose-dependent, non-additive gene expression changes are consistent with the reported non-additive effects on RLW, hepatocellular fat accumulation, and PCB153 tissue levels. Funded by SBRP P42ES04911.

PS 2306 DETERMINING A ROBUST D-OPTIMAL DESIGN FOR TESTING FOR DEPARTURE FROM ADDITIVITY IN A MIXTURE OF FOUR PFAAS.

C. Carr[1], C. Gennings[1], B. D. Abbott[2], J. E. Schmid[2], W. Wan[1], L. Burgoon[2], C. J. Wolf[2] and C. Lau[2]. [1]*Biostatistics, Virginia Commonwealth University, Richmond, VA and* [2]*NHEERL, U.S. EPA, Research Triangle Park, NC.*

Our objective is to determine an optimal experimental design for a mixture of perfluoroalkyl acids (PFAAs) that is robust to the assumption of additivity. PFAAs are widely used in consumer products and industrial applications. The presence and persistence of PFAAs, especially in human body fluids, have raised concern about this class of compounds. Of particular focus to this research project is whether an environmentally relevant mixture of four PFAAs with long half-lives (in years: PFOA, PFOS, PFNA, and PFHxS) act synergistically. PFAAs are thought to activate peroxisome proliferator-activated receptor alpha (PPARα) to produce liver toxicity and developmental effects. The first phase of study included evaluating the ability of PFAAs to bind PPARα in in vitro studies of a transfected-cell model. Using the resulting data, a non-linear logistic additivity model was employed to predict relative luciferase units (RLU) at an environmentally relevant mixing ratio. The mixing ratio was estimated from NHANES (2005-2006) data. We used a maximin D-optimal design criterion to select from a large set of specified designs. The candidate seven point designs (control plus six dose groups) were generated by varying the total dose locations and shape of the dose-response curve with the additivity curve used as a reference. A total of 6,006 designs were considered with seven dose groups along with 13 different dose-response shapes. A D-optimal design (i.e., the location of the seven dose groups) robust to misspecification of the resulting dose-response shape was found with a minimum D-efficiency of 92%. The proposed design has good statistical properties over a band of possible dose-response curves including synergistic, additive and antagonistic associations among the four PFAAs. This research was partially supported by NIEHS T32ES007334 and does not reflect USEPA policy.)

PS 2307 PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING OF TWO BINARY MIXTURES: METABOLIC ACTIVATION OF CARBON TETRACHLORIDE BY TRICHLOROETHYLENE AND METABOLIC INHIBITION OF CHLOROFORM BY TRICHLOROETHYLENE.

M. V. Evans[1], H. M. Yang[2], K. A. Yokley[3], A. McDonald[1], Y. M. Sey[1], C. R. Eklund[1] and J. Simmons[1]. [1]*NHEERL/ORD, U.S. EPA, Research Triangle Park, NC,* [2]*NCEA/ORD, U.S. EPA, Washington, DC and* [3]*Mathematics and Statistics, Elon University, Elon, NC.*

The interaction between trichloroethylene (TCE) and chloroform (CHCl3) has been described as less than additive, with co-exposure to TCE and CHCl3 resulting in less hepatic and renal toxicity than observed with CHCl3 alone. In contrast, the nonadditive interaction between TCE and carbon tetrachloride (CCl4) results in increased hepatotoxicity when compared with CCl4 alone. To explore the mode of action underlying these nonadditive interactions, male F-344 rats were exposed in vapor uptake inhalation chambers to TCE alone, CHCl3 alone, CCl4 alone, both TCE and CHCl3 or both TCE and CCl4. Metabolic inhibition was observed as a slower rate of decay in the vapor uptake data, while increased decay in the data represented metabolic activation. In the presence of TCE, uptake of CHCl3 decreased while uptake of CCl4 increased. Physiologically-based pharmacokinetic (PBPK) models for each chemical alone were developed and used to construct and evaluate PBPK models describing the influence of concurrent exposure to TCE on the uptake and metabolism of CHCl3 or CCl4 as well as the influence of concurrent exposure to CHCl3 or CCl4 on clearance of TCE. The model simulations indicated that TCE had opposing effects on the metabolism of CHCl3 and CCl4, decreasing the metabolism of CHCl3 and increasing the metabolism of CCl4. Computer simulations of the various types of metabolic inhibition suggest competitive inhibition best describes the metabolic interaction between TCE and CHCl3. For TCE and CCl4, a novel metabolic hypothesis describing activation of CCl4 by TCE best fit the data. In summary, different modes of action for the nonadditive interactions resulting from co-exposure to TCE and CHCl3 and from co-exposure to TCE and CCl4 have been suggested by PBPK modeling. (This abstract does not reflect EPA policy.)

PS 2308 INTEGRATED MULTI-DISCIPLINARY ASSESSMENT OF ENVIRONMENTALLY REALISTIC COMPLEX MIXTURES OF DRINKING WATER DISINFECTION BY-PRODUCTS (DBPS) (THE 4LAB STUDY).

J. Simmons[1], M. G. Narotsky[1], L. K. Teuschler[2], J. G. Pressman[3], E. S. Hunter[1], G. E. Rice[2], G. R. Klinefelter[1], J. M. Goldman[1], T. F. Speth[3], L. F. Strader[1], R. J. Miltner[3], S. Parvez[2], A. McDonald[1], D. S. Best[1], C. A. Dingus[4] and S. D. Richardson[5]. [1]*NHEERL/ORD, U.S. EPA, Research Triangle Park, NC,* [2]*NCEAORD, U.S. EPA, Cincinnati, OH,* [3]*NRMRL/ORD, U.S. EPA, Cincinnati, OH,* [4]*Battelle, Columbus, OH and* [5]*NERL/ORD, U.S. EPA, Athens, GA.*

More than 600 DBPs have been identified; yet ~50% of the total organic halide from chlorination is unidentified. Epidemiology studies suggest associations between human use of chlorinated water and reproductive/developmental effects

(pregnancy loss, low birth weight), that are unexpected based on available single-chemical animal data. To evaluate pup weight and prenatal loss, and other endpoints, we conducted a multigenerational reproductive/developmental assay, integrated with extensive chemical analysis. To evaluate DBPs at environmentally relevant doses and include the large unidentified fraction, methods were developed for: statistical power; concentration and chlorination of water concentrates; an animal water delivery system; and, risk assessment. Source water was concentrated ~130 fold by reverse osmosis, chlorinated, and provided as drinking water to Sprague-Dawley rats; controls received purified water. Timed-pregnant females (P0 generation, 40 control:60 treated) were exposed from gestation day 2 until weaning of the F1 litters. Treatment of F1 animals continued throughout the study. A second block of P0 females was maintained through PD 6 of the F1 generation. No treatment effects were detected for pup weight, pre- and post-natal loss, eye opening, gestation length, nipple retention, or body weight. In F1 adults, no effects were detected on organ weights, male serum testosterone or female hypothalamic catecholamine levels. Statistically significant effects were increased anogenital distance in F1 males and a slight (<1 day) delay in puberty in F1 females. The delayed puberty was consistent with the dose-response of a defined mixture of 9 regulated DBPs mixed at a representative ratio. (Abstract does not reflect EPA policy.)

PS 2309 A FOUR-STEP APPROACH FOR EVALUATION OF DOSE-ADDITIVITY.

R. C. Hertzberg[1], Y. Pan[2], R. Li[2], L. T. Haber[3], R. Lyles[2], V. C. Moser[4], D. W. Herr[4] and J. Simmons[4]. *[1]Biomathematics Consulting, Atlanta, GA, [2]Department of Statistics, Emory University, Atlanta, GA, [3]Toxicology Excellence for Risk Assessment, Atlanta, GA and [4]NHEERL, U.S. EPA, Research Triangle Park, NC.*

A four-step approach was developed for evaluation of chemical mixture data for consistency with dose addition. Following the concepts in the U.S. EPA mixture guidance (EPA 2000), toxicologic interaction for a defined mixture (all components known) is departure from a clearly articulated definition of component additivity. The EPA guidance specifies two key characteristics of dose-additivity: 1) the mixture components have toxic potencies that are proportional to each other, and 2) the mixture dose can be represented by a linear combination of the component doses. The component chemicals must then share a common dose-response model, where only the dose coefficients depend on the chemical. We define this as the combined prediction model (CPM). Further, the mixture must be described by the same model type, with a distinct coefficient for the total mixture dose. Consequently, the mixture response can be predicted from the component dose-response curves by using the CPM with dose as the linear combination of component doses. The four steps are to evaluate: 1) toxic proportionality by determining how well the CPM matches the single chemical models; 2) fit of the mixture model (the model developed using just the mixture data) to the mixture data; 3) agreement between the mixture data and the CPM; and 4) consistency between the CPM and the mixture model. These four steps contribute different pieces of information about the consistency of the component chemical and mixture dose-response data with this two-part definition of dose-additivity. Because there are four evaluations instead of one, there are more opportunities to reject dose-addition, so statistical adjustment for multiple comparisons is necessary, and interpretation of the toxicologic significance of the results is critical. This approach is demonstrated with neurotoxicity data on carbamate mixtures. (This abstract does not reflect EPA policy.)

PS 2310 TOXICITY OF TUNGSTEN AND COBALT MIXTURES IN EMBRYONIC ZEBRAFISH.

J. L. Freeman, J. M. Schultheis, D. R. Robbins and S. M. Peterson. *Health Sciences, Purdue University, West Lafayette, IN.*

Tungsten (W) is a heavy metal that is used abundantly in industrial applications and was implemented as a nontoxic substitute to lead in ammunition in the mid-1990s. Since this time, W has been found to be able to move into groundwater sources and was reported to be associated with childhood leukemia clusters in the Western US. These findings have raised questions about W exposure and public safety. Recent studies suggest that W exposure alone may have limited toxicity but that there is potential synergistic toxicity when present in mixtures with other heavy metals. In this study, the potential synergistic effects of W (as sodium tungstate) and cobalt (Co; as cobalt chloride) were investigated in vivo in developing zebrafish. An acute toxicity test was first performed for each metal on a range of concentrations to measure effect levels on hatching rates and survival from ~2 through 120 hours post fertilization (hpf). Significant mortality was observed only at the highest W concentration (100 parts per thousand; ppt) and was significant as soon as 12 hpf. The hatching rate was only negatively affected at this highest concentration. In the Co test, significant mortality was observed only at the highest concentration (100 parts per million; ppm) at the last three time points. A slight hatching delay was observed at 54 hpf in the two highest concentrations of Co (10 and 100 ppm). The potential synergistic toxicity of W/Co in mixture was assessed at concentrations based on the above mentioned findings to determine if Co influenced W toxicity. The most significant finding was in the mixture containing the highest concentrations of W (10 ppt) and Co (10 ppm), where a delay in mortality was observed in comparison to embryos exposed to W alone at the same concentration and in mixture with a lower concentration of Co (1 ppm). From the range of concentrations included in this test, our results showed an opposite effect than expected in that the mixture including the highest concentration of Co delayed the toxicity of the highest W concentration.

PS 2311 SIMULTANEOUS EXTRACTION AND DETECTION OF BISPHENOL A AND GENISTEIN.

J. Coughlin[1, 2, 3] and B. Buckley[1, 2, 3]. *[1]Toxicology, Rutgers University, Piscataway, NJ, [2]JGPT, Rutgers University, Piscataway, NJ and [3]Environmental and Occupational Health Sciences Institute, Rutgers University, Piscataway, NJ.*

Bisphenol A (BPA) is a synthetic monomer used in the production of polycarbonate plastics and epoxy resins. A common component of food and beverage containers, BPA can leach from containers into contents, directly exposing humans to BPA. Genistein is a natural phytoestrogen abundant in soy products such as soy-based infant formula. There have been a growing number of analyses on the effects of BPA and genistein due to increasing evidence of their endocrine disrupting properties. Some studies report beneficial effects of genistein while others show adverse effects on development and reproduction. Because BPA and genistein share common pathways for metabolism, potential interactions between these pathways during co-exposures of BPA and genistein could result in more pronounced effects. Such metabolomic investigations necessitated the development of a method to simultaneously extract and detect BPA and genistein. We devised a co-elution assay in which BPA and genistein were extracted from spiked rat plasma via solid phase extraction (SPE). Three SPE cartridges were tested and three elution paradigms were employed. Overall recoveries of BPA and genistein ranged from 54.88-122.01% and 5.57-139.93%, respectively. Recoveries were optimal when extracted from Bond Elut Plexa cartridges using methanol and acetonitrile, with average recoveries of 75.46% and 100.59% for BPA and genistein, respectively. Identification and quantification of BPA and genistein were performed with a validated liquid chromatography-tandem mass spectrometry (LC-MS-MS) method that utilizes ion-trap technology with electrospray ionization (ESI) and selective reaction monitoring (SRM). Detection limits for genistein and BPA were 16.5 and 100 picograms, respectively. In summary, we have developed a co-elution method and a sensitive quantitative assay which should be amenable to the detection of BPA and genistein in urine, blood, and tissue following in vivo exposures. Supported by EOHSI, T32ES007148, NIEHS ES05022.

PS 2312 REACTIVITY OF SELECTED OSTEOLATHYROGENS WITH A MODEL COFACTOR FOR LYSYL OXIDASE.

H. Bensinger[1], N. Genco[1], R. G. Bergosh[2] and D. A. Dawson[1].
[1]Biology/Toxicology, Ashland University, Ashland, OH and [2]Chemistry, Geology, and Physics, Ashland University, Ashland, OH. Sponsor: D. Fort.

Osteolathyrogens disrupt the normal connective tissue fiber cross-linking process, leading to connective tissue fragility. Normal cross-linking relies, in part; on lysyl oxidase (LO) to catalyze an oxidation reaction that eventually gives rise to insoluble fibers. Lysyl tyrosine quinone (LTQ) serves as an important cofactor for LO in this process. Chemicals from a variety of structural classes have been shown to induce osteolathyrism, including certain aminonitriles, ureides, acid and benzoic acid hydrazides, carbazates, and dithiocarbamates. Previous work has shown that some osteolathyrogens have the ability to react with 4-butylamino-5-methyl-o-quinone (mLTQ), an agent developed as a model cofactor for LO. In this study mLTQ was synthesized and its structure verified using NMR spectroscopy. Relative reactivity of mLTQ was determined using UV-vis scanning spectrophotometry through sequential addition of 1 mM (final concentration) of an individual osteolathyrogen every 10 min to reach a final concentration of 20 mM. Three previously tested osteolathyrogens were re-evaluated with mLTQ and the results confirmed the earlier findings. Osteolathyrogen-mLTQ reactivity was then assessed for eight additional agents. Overall three patterns of reactivity were observed: 1) rapid reactivity (<20 min) with adduct formation at ~350 nm and simultaneous bleaching of the mLTQ peak at ~500 nm, 2) rapid reactivity with adduct formation (at ~350 nm) but without bleaching (at ~500 nm), and 3) very slow reactivity (>24 hr) with adduct formation and bleaching. These results, when coupled with data available from frog embryo mixture toxicity studies, offer the opportunity to develop a more definitive assessment of mechanisms of action for osteolathyrism.

PS 2313 THE SYNERGISTIC EFFECT OF SODIUM CHLORITE AND BROMOCHLOROACETIC ACID ON BROMATE-INDUCED RENAL CELL DEATH.

X. Zhang[1], R. J. Bull[2], J. A. Cotruvo[3], J. Fisher[4] and B. S. Cummings[1]. *[1]Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA, [2]MoBull Consulting, Richland, WA, [3]Joseph Cotruvo & Associates, LLC, Washington, DC and [4]National Center for Toxicological Research, U.S. FDA, Jefferson, AR.*

Our previous study demonstrated that bromate, a drinking water disinfection by-product, induced renal cell death via DNA-dependent and independent mechanisms. In addition to bromate, drinking water contains other disinfection by-products, which may work in tandem to enhance cytotoxicity. Therefore, we tested the combined effect of two of these by-products, sodium chlorite and bromochloroacetic acid (BCAA) on bromate cytotoxicity in normal rat kidney (NRK) cells. Sodium chlorite and BCAA alone induced cytotoxicity at concentrations of 20 and 50 ppm, respectively, while bromate was only moderately cytotoxic at concentrations of 200 ppm. Combining bromate with sodium chlorite or BCAA alone enhanced cytotoxicity 1.5-4 folds. Exposing cells to all three compounds simultaneously resulted in synergistic-like increases in cytotoxicity. This effect did not correlate to increases in reactive oxygen species (ROS), even though all three compounds induced ROS formation alone. Sodium chlorite, but not BCAA increased bromate-mediated DNA damaged as measured by 8-hydroxydeoxyguanosine staining. In addtion, sodium chlorite, other than BCAA, partially reversed bromate-induced G2/M cell cycle arrest. Both compounds increased apoptosis as assessed by annexin V, PI, and DAPI staining. This is in direct contrast to bromate treatment alone which induced necrosis. Western blot results showed that both sodium chlorite and BCAA increased bromate-induced histone H2AX phosphorylation, a marker of DNA damage, and p38 MAPK phosphorylation, but only BCAA increased bromate-induced phosphorylation of p53. Collectively, these data suggest that sodium chlorite potentiates bromate-induced renal toxicity by enhancing DNA damage; while BCAA may increase toxicity using pathways independent of DNA damage.

PS 2314 CELL SIGNAL TRANSDUCTION TO PREDICT MIXTURE INTERACTIONS.

J. Boyd, X. Gao, H. Williams, C. Kinzer and J. Vrana. *Bennett Department of Chemistry, West Virginia University, Morgantown, WV.*

While exposure to chemical mixtures is an everyday reality, an understanding of their combined effects, and any potential prediction thereof, is extremely limited. Realistic exposures potentially consist of hundreds to thousands of chemicals per day, but even relatively simple binary mixture interactions can be inherently difficult to predict based upon the lack of temporal and spatial mechanisms for the individual constituents. To this end my laboratory has been developing in vitro assays to enable a high-throughput means of defining general toxicodynamic response pathways in vitro, with a full dosing regimen, which has the potential to identify and predict possible interactions. A central aspect of our assays elucidates altered activity for cell signaling cascades following multiple time-course exposures to toxicants which allows for the identification of both their common and disparate response pathways. Preliminary results will be presented with data gathered from hepatocytes exposed to deguelin (0.001-100 uM), potassium cyanide (0.001-100 uM), staurosporine (10 uM) and SB202190 (350 nM), alone and in combination, for 24 hours. Oxygen consumption kinetic profiles suggested key changes in ATP production at 400 minutes post-dose which initiated an investigation into the activity of the signaling cascades via a bead-based multiplexed (8-plex) immunoassay at that time point. Dose-dependent cascade initiation indicates a clear identification of threshold response to low-level exposures, and crosstalk amongst selected proteins correctly predicts mixtures interactions. In this study, we demonstrate the potential of a new in vitro approach for the prediction of toxic mixtures interactions that is fundamentally driven by the interdependence of energy metabolism, signal transduction, and cell survival.

PS 2315 PIFITHRIN-α INCREASES IGF-1R INHIBITOR MEDIATED HEPG2 HEPATOCARCINOMA DEATH.

X. Gao, H. Williams, C. Kinzer, J. Vrana and J. Boyd. *Bennett Department of Chemistry, West Virginia University, Morgantown, WV.*

Insulin-like growth factor receptor I (IGF-1R) has been shown to be crucial for tumor transformation, and it expression has been related to p53 activity. Conversely, p53 expression in response to DNA damage may be regulated by IGF-1R. Additionally, p53 has been shown to have regulatory control over mitochondrial respiration which can lead to an increase in the reliance on glycolysis for energy metabolism in cancer cells. We have investigated the effect of a p53 inhibitor, Pifithrin-α, and an IGF-1R inhibitor, AG1024, on HepG2 hepatocarcinoma cells. Briefly, we measured oxygen consumption, glucose uptake, and viability of HepG2 cells treated with Pifithrin-α and AG1024 (individually or in combination) in the presence and absence of glucose. Our findings show that Pifithrin-induced HepG2 cytotoxicity is independent of the presence or absence of glucose. However we also found that Pifithrin decreases oxygen consumption in glucose-rich media, but increases oxygen consumption in its absence. Further, the viability of HepG2 cells in response to AG1024 is greatly decreased in the absence of glucose, while oxygen consumption remains relatively stable. Additionally, we have found that combinations of the inhibitors appear additive in presence of glucose, but result in greater than additive toxicity in the absence of glucose. Overall, our findings strengthen the case for the presence of a glycolytic modulator upstream of p53, but downstream of IGF-1R signaling in this type of cancer. Understanding the relationship between IGF-1R, p53, and energy metabolism may help elucidate their role in carcinogenesis, and further define additional mechanisms that may enhance chemotherapeutic discovery.

PS 2316 PBPK MODELING OF THE AGGREGATE AND CUMULATIVE EXPOSURES OF RATS TO TOLUENE, N-HEXANE, CYCLOHEXANE, AND ISOOCTANE.

N. Kaveh, M. Gagné, G. Charest-Tardif, R. Tardif and K. Krishnan. *Université de Montréal, Montreal, QC, Canada.*

Mixture risk assessments focusing on both the aggregate and cumulative exposures can be improved with an understanding of the change in internal dose. The modeling of the internal dose and pharmacokinetics of hydrocarbons following cumulative and aggregate exposures has not yet been undertaken. The objective of this study was to develop a PBPK model to simulate the mixed exposure of rats to four hydrocarbons (toluene (T), n-hexane (H), cyclohexane (C) and isooctane (I); chosen to represent chemical classes in large volume petroleum products (e.g., gasoline, kerosene, gas oils)) by inhalation and oral routes. The PBPK models of individual chemicals were initially developed, and then were interconnected using the metabolic interaction terms. The resulting model was first used to simulate the kinetics of each chemical following a single route of exposure, and then to simulate the data collected following multiroute exposure. The capacity of the PBPK model to adequately simulate the kinetics following aggregate and cumulative exposures was evaluated by comparing with the experimental data. These data were collected in groups of male Sprague-Dawley rats (n=5) exposed either to a single dose of each substance by inhalation (50 ppm of T and H; 300 ppm of C and I; 2-hr) or oral gavage (8.3, 5.5, 27.9, and 41.27 mg/kg, respectively, for T, H, C and I) or to multi-products given by both routes together at the same dose levels. The data-derived area under the blood concentration vs time curve (AUC; mg/L x hr) were 106.62, 12.97, 117.56 and 122.27 whereas the AUC values derived from the multiroute, multichemical PBPK model were: 114.04, 12.68, 58.99, and 123.42 mg/L x hr, respectively, for T, H, C and I. Overall, the PBPK model developed in this study is a useful tool for simulating kinetics of hydrocarbons, by accounting for not only the saturable metabolism but also for the interactive effects during aggregate and cumulative exposures.

PS 2317 NAIL MANGANESE AS A BIOMARKER OF WELDING FUME EXPOSURE.

J. M. Antonini, J. R. Roberts and K. Sriram. *NIOSH, Morgantown, WV.*

Manganese (Mn) is a common metal in welding fume aerosols and suspected of inducing adverse neurological effects in exposed workers. Measurements of Mn in blood and urine have proven to be unreliable indicators of exposure. The goal of the current study was to determine if Mn accumulation in the nail (claw) can be used as a potential biomarker. To model this, male Sprague-Dawley rats were treated by intratracheal instillation (IT) with 2 mg/rat of shielded manual metal arc-hardsurfacing (MMA-HS; high Mn) or gas metal arc -mild steel (GMA-MS; low Mn) welding fumes once a week for 28 weeks. Vehicle controls received saline by IT. The percentage of Mn, relative to the other metals in the collected fume, was more than double (50.9 %) in the MMA-HS fume compared to GMA-MS (21.7 %). At 7 days after the last exposure, right lung lobes, specific brain regions, blood, and nails were harvested from each group. Mn levels were determined by inductively coupled plasma atomic emission spectroscopy. Significant elevations in lung Mn were observed following repeated exposure to MMA-HS and GMA-MS fumes for 28 weeks. Despite the difference in Mn composition of the two fumes, repeated exposure to equal mass doses of the fumes produced comparable Mn lung burden, suggesting a steady-state may have been achieved. Measurements of blood Mn were near or at the limit of detection and, as a consequence, variable. In contrast, Mn

levels significantly increased in the nails, as well as in striatum and midbrain, potential targets of Mn neurotoxicity, for the MMA-HS group compared to the GMA-MS group and controls. Our results demonstrate nail Mn to be a potentially viable and sensitive biomarker for welding fume exposure. The ease with which nails can be harvested, transported, and stored makes them an attractive surrogate for monitoring exposures in occupational settings.

PS 2318 PERIPHERAL BLOOD GENE EXPRESSION PROFILING REVEALS SILICA-INDUCED PULMONARY TOXICITY.

R. Sellamuthu, C. Umbright, J. Roberts, R. Chapman, S. Young, D. Richardson, D. Leonard, W. McKinney, B. Chen, D. Frazer, S. Li, M. Kashon and P. Joseph. *National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV.*

The present research aimed to investigate peripheral blood gene expression profiling as a minimally invasive surrogate approach to study silica-induced pulmonary toxicity. Rats were exposed to crystalline silica by inhalation (15 mg/m3, 6 hours/day, 5 days). Pulmonary damage and blood gene expression profiles were determined at various latency periods (0 - 16 weeks). Silica exposure resulted in pulmonary toxicity in the rats as evidenced by histological changes in the lungs and elevation of LDH activity in the bronchoalveolar lavage fluid (BALF). Analysis of global gene expression profiles in the blood of the rats identified genes that were differentially expressed in response to silica exposure. The differential blood gene expression profiles correlated with the pulmonary toxicity parameters in the silica exposed rats. Genes involved in biological functions such as inflammatory response, cancer, pulmonary damage, oxidative stress, energy metabolism, fibrosis, etc, were found differentially expressed in the blood of the silica exposed rats compared with the controls. Induction of pulmonary inflammation in the silica exposed rats, as suggested by differential expression of inflammatory response genes in the blood, was supported by significant increases in the number of neutrophils and macrophages as well as the activity of pro-inflammatory chemokines – MCP1 and MIP2, observed in the BALF of the silica exposed rats. A silica-responsive blood gene expression signature developed using the gene expression data predicted with significant accuracy the exposure of rats to lower concentrations (1 and 2 mg/m3) of silica. Taken together our findings suggest the potential application of peripheral blood gene expression profiling as an efficient surrogate approach to study silica-induced pulmonary toxicity.
Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by NIOSH and should not be constructed to represent any agency determination or policy.

PS 2319 BIOACTIVATION OF THE NASAL TOXICANT 2, 6-DICHLOROBENZONITRILE: AN ASSESSMENT OF METABOLIC ACTIVITY IN HUMAN NASAL MUCOSA AND IDENTIFICATION OF BIOMARKERS OF EXPOSURE AND POTENTIAL TOXICITY.

F. Xie[1,2], X. Zhou[1], J. D'Agostino[1], D. Spink[1] and X. Ding[1]. [1]*Wadsworth Center, New York State Department of Health, Albany, NY and* [2]*School of Public Health, State University of New York at Albany, Albany, NY.*

The herbicide 2,6-dichlorobenzonitrile (DCBN) is a potent nasal toxicant in rodents. However, it remains unknown whether DCBN causes nasal toxicity in humans. CYP2A5, a P450 enzyme predominantly expressed in mouse nasal mucosa, is largely responsible for converting DCBN into electrophilic intermediates, which, through formation of glutathione conjugates (DCBN-GS) and protein adducts, cause tissue damage. The human orthologs of CYP2A5, CYP2A6 and CYP2A13, are both expressed in the nasal mucosa, and are capable of activating DCBN. Therefore, we hypothesized that 1) human nasal tissues can activate DCBN; and 2) DCBN-GS or its derivatives can serve as biomarkers of potential DCBN exposure and toxicity. To test these hypotheses, we first established a sensitive LC-MS/MS method for detection and quantification of DCBN-GS in biological matrices. We then demonstrated that human fetal nasal mucosa microsomes catalyzed the formation of DCBN-GS *in vitro*, with a Km value comparable to that for DCBN-GS formation in adult mouse nasal microsomes. The involvement of CYP2A enzymes in this bioactivation in human nasal microsomes was confirmed by the finding of an inhibition of the activity by 8-methoxyporsalen, a known CYP2A inhibitor. Furthermore, DCBN-GS was detected in the nasal mucosa and nasal-wash fluid obtained from DCBN-exposed mice; the detected amounts of DCBN-GS increased, with increases in DCBN dose, and the conjugate was detectable at as early as 30 min after DCBN exposure. Moreover, metabolites of DCBN-GS, including DCBN-Cys, which may serve as alternative biomarkers, were also detected in the nasal mucosa and nasal-wash fluid. Thus, our data indicate that DCBN is potentially toxic to human nasal tissues, and that DCBN-GS (or its derivatives) in nasal-wash fluid may serve as biomarkers of DCBN exposure and potential nasal toxicity in humans. (Supported in part by NIH grant ES007462)

PS 2320 SYSTEMIC UPTAKE OF ^{14}C 2, 3-BUTANEDIONE ADMINISTERED BY INTRATRACHEAL INSTILLATION IN MALE SPRAGUE-DAWLEY RATS AND OROPHARYNGEAL ASPIRATION IN MALE B6C3F1 MICE.

T. Fennell[1], D. Morgan[2], S. Watson[1] and S. Waidyanatha[2]. [1]*RTI International, Research Triangle Park, NC and* [2]*National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

2,3-Butanedione (BD) is a reactive diketone that is found in butter and has been used in artificial butter flavor, and is thought to cause bronchiolitis obliterans in popcorn workers. The extent of uptake of BD from inhalation exposure is a concern. The objective of this study was to evaluate the ability of BD to be taken up in the lung, enter the systemic circulation, and bind to hemoglobin and albumin. [1-^{14}C] BD was administered to male Sprague Dawley rats (100 mg/kg) by intratracheal instillation (ITI), and to male B6C3F1 mice (200 mg/kg) by oropharyngeal aspiration (OPA). After 24 h, animals were euthanized and blood collected. Blood and plasma were analyzed for ^{14}C to estimate the systemic dose at 24h. Binding to plasma albumin was assessed following isolation by trichloroacetic acid precipitation, and ultrafiltration, or by ammonium sulfate precipitation. Binding to hemoglobin was assessed by dialysis of hemolysate followed by size exclusion HPLC, or by precipitation of globin from hemolysate with acidic acetone. At 24 h, 1.2 ± 0.1 % of the dose was found in rat blood, 0.66 ± 0.06 % in rat plasma, 0.35 ± 0.12 % in mouse blood and 0.17 ± 0.05 % in mouse plasma. Albumin binding in rats was 3.12 ± 0.28 nmol equiv/mg albumin, with 38% of the radioactivity in plasma bound to albumin. In mice, binding was 0.99 ± 0.26 nmol equiv./mg albumin, with 45% of the radioactivity in mouse plasma bound to albumin. The extent of binding to hemoglobin in the rat was 0.44 ± 0.20 nmol equiv/mg hemoglobin, and 0.31 ± 0.05 nmol/mg globin. In mice, the extent of binding to hemoglobin was 0.19 ± 0.10 nmol/mg. This study demonstrated that BD, following administration by ITI in rats or by OPA in mice, can enter the systemic circulation and react with hemoglobin and albumin. This shows the promise for evaluating hemoglobin and albumin adducts as biomarkers of exposure in humans.

PS 2321 RESVERATROL, AN AHR LIGAND, ALLEVIATES BACTERIAL ENTEROTOXIN-INDUCED ACUTE LUNG INJURY *VIA* UPREGULATION OF MIRNA 155 AND SUBSEQUENT INCREASE IN MYELOID-DERIVED SUPPRESSOR CELLS.

S. A. Rieder, P. Nagarkatti and M. Nagarkatti. *Pathology Microbiology and Immunology, University of South Carolina, Columbia, SC.*

Acute lung injury (ALI) has many etiologies such as inhalation of a toxic substance, pneumonia, surgery and sepsis. The treatment options for ALI are very limited, and better therapeutic options are urgently needed. We administered Staphylococcal enterotoxin B (SEB, 50μg/mouse) through the intranasal route in order to induce ALI, and treated mice with resveratrol (RES), a natural plant product and an AhR ligand. We demonstrated that pre-treatment with 100 mg/kg bodyweight oral RES alleviated vascular leak and edema induced by SEB. SEB inhalation resulted in a significant increase in the percentage and absolute cell numbers of Gr-1+CD11b+ Myeloid Derived Suppressor Cells (MDSCs) in the lungs. Moreover, resveratrol pre-treatment further increased the percentage and absolute number of MDSCs. Arginase expression, a hallmark of MDSCs, was significantly increased in RES-treated mice when compared to vehicle controls. While SEB activation in vitro resulted in extensive T cell proliferation, addition of sorted MDSCs, particularly those from RES-treated groups, caused significant suppression of T cell expansion. Next, we carried out an affymetrix survey of 600 different miRNAs in lung infiltrating lymphocytes. Ingenuity pathway software analysis revealed 11 important miRNAs that were differentially regulated in SEB-administered mice versus RES-treated groups. Interestingly, miRNA 155 exhibited 18.764 fold change in expression in SEB+vehicle-administered group when compared to 46.474 fold change in SEB+RES-treated group. We also studied the target genes of miRNA 155, and found that IL-1β and NOS-2 were highly upregulated in SEB-administered group compared to the control. RES treatment further increased the expression of these

genes that play a crucial role in MDSC development and function. In conclusion, resveratrol treatment leading to activation of AhR, aids in the alleviation of disease by upregulating miRNA 155 and subsequently increasing MDSCs in the lungs.

PS 2322 MOLECULAR PATHWAYS OF PULMONARY INFLAMMATION FOLLOWING ASPIRATION AND INHALATION OF STAINLESS STEEL WELDING FUME IN MICE.

P. C. Zeidler-Erdely, A. Erdely, M. Kashon, S. Li and J. Antonini. *HELD, NIOSH, Morgantown, WV.*

Previously, at comparable doses, we observed a greater inflammatory potency of inhaled versus aspirated gas metal arc-stainless steel (GMA-SS) welding fume in C57BL/6J (B6) mice. Also, aspiration of GMA-SS fume provoked a transient inflammation which resolved by 7d while inhalation resulted in a mounting response that remained unresolved at 28d post-exposure. Furthermore, we found by 28d after aspiration, lung gene expression was down-regulated which confirmed that inflammation was resolved. Here, we examined the lung transcriptional response after inhalation of GMA-SS fume and used gene network-based analysis to compare with previous aspiration data. Mice were exposed to GMA-SS fume at 40mg/m3 x 3hr/d for 10d. Necropsy was done at 28d after the last exposure and whole lung microarray was performed. A core analysis in IPA 8.7 was done on the inhalation data ($p<0.05$; fold change >1.5) followed by a core comparison analysis of the inhalation and aspiration datasets. We found that inhalation of GMA-SS welding fume was associated with activation of complement (C2, C3, C1QA-C, C4B), type 1 interferon pathways and increased expression of monocyte and lymphocyte chemotactic genes such as CCL2, CCL7 and CCL8. In addition, genes encoding the expression of molecules involved in T lymphocyte and natural killer cell regulation were increased (CD86 and CD69). Upon comparison of the datasets, a greater number of genes were changed with inhalation versus aspiration exposure. Involvement of IL1B as a central mediator to the lung response was apparent between the different exposure regimes as well as increased expression of MMP12. Transcriptional regulation of the lung response to GMA-SS aspiration involved FOS, EGR1, FOSB. In contrast, IRF7 and 9 as well as STAT1 and 2 were involved with inhalation. Overall, gene expression was reflective of the unresolved inflammation at 28d after inhalation of GMA-SS fumes. With the exception of a few molecules, differences in gene network signatures are apparent between aspiration and inhalation of GMA-SS welding fume in B6 mice.

PS 2323 EFFECTS OF PARTICULATE AIR POLLUTION DURING BEIJING OLYMPIC GAMES IN A MOUSE MODEL.

K. Tzan[1], X. Xu[1], S. Jiang[1], J. Aronovsky[1], A. Wang[2], S. Rajagopalan[2, 3] and Q. Sun[1, 2, 3]. [1]*Public Health, The Ohio State University, Columbus, OH,* [2]*Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH and* [3]*Cardiology, The Ohio State University, Columbus, OH.*

Many studies have linked ambient particulate matter air pollution to increased morbidity and mortality of cardiovascular diseases in the general population, but the biological mechanisms of these associations have yet to be elucidated. This study intended to take advantage of drastic intervention in air quality improvement from local authorities during the 2008 Beijing Olympic Games and attempted to quantify the effects of ambient fine particulate matter (diameter < 2.5μm, PM2.5) on inflammatory responses in mice during and after the Games. C57BL/6 male mice at age week 8 were exposed to either ambient PM2.5 or filtered air (FA) at an exposure location 4 miles from the Olympic Center during and after the Games for 2 months, respectively. During the 2-month Olympic Games, the average PM2.5 was 60.79 μg/m3. The PM2.5 rose to an average of 70.21 μg/m3 during the 2-month time period after the Games. During the Games, circulating monocyte chemoattractant protein (MCP-1) and interleukin-6 were remarkably increased by PM2.5 exposure when compared to the FA group. Additionally, macrophage count in the lung and adipose tissue (epididymal) was significantly higher in the PM2.5 group. After the Olympic Games, neutrophils were markedly higher in the spleen in the PM2.5 group. The macrophage count was also significantly elevated in the lung and adipose tissue. In a comparison of PM2.5 exposure between the two time periods, macrophage, neutrophil, and lymphocyte infiltrations in the lung were significantly increased in the groups after the Olympic Games, along with significant increase of macrophage in the adipose tissue. These data show that particulate air pollution induced a significant increase of inflammatory cell infiltration in pulmonary and adipose tissues, suggesting that air quality improvement can reduce inflammatory response systemically and can be beneficial to inflammation-associated diseases.

PS 2324 OVER-EXPRESSION OF THE NF-κB MEMBER RELB DAMPENS THE PRO-INFLAMMATORY EFFECTS OF LONG-TERM CIGARETTE SMOKE EXPOSURE.

D. McMillan[1, 3], T. H. Thatcher[3, 4], S. Maggirwar[2], P. J. Sime[1, 3, 4] and R. P. Phipps[1, 2, 3]. [1]*Environmental Medicine, University of Rochester, Rochester, NY,* [2]*Microbiology and Immunology, University of Rochester, Rochester, NY,* [3]*Lung Biology and Disease, University of Rochester, Rochester, NY and* [4]*Pulmonary and Critical Care Medicine, University of Rochester, Rochester, NY.*

Rationale: The NF-kB family member RelB attenuates cigarette smoke-induced inflammation in lung fibroblasts. RelB-deficient mice are more susceptible to inflammation than wild-type mice. We have recently demonstrated that RelB over-expression in mouse airways prior to acute cigarette smoke exposure dampens smoke-induced inflammation. We hypothesized that RelB over-expression would dampen pre-existing inflammation resulting from long-term smoke exposure.
Methods: Female C57BL/6 mice received long-term regimens of cigarette smoke to induce inflammation. Following this, mice were treated with two doses of a RelB recombinant adenovirus by intranasal aspiration over 24 hours to over-express RelB in mouse airway cells. Control mice received equal doses of filtered air and of a control adenovirus. Bronchoalveolar lavage (BAL) fluid was collected for ELISA and differential cell counts. Lungs were harvested for Western blot, immunohistochemistry, and detection of myeloperoxidase activity.
Results: RelB over-expression decreases cigarette smoke-induced neutrophil infiltration into the bronchoalveolar space. Production of prostaglandin E2 (PGE2) and pro-inflammatory cytokines, including IL-6, MIP-2 and KC, are also reduced following RelB over-expression. Cyclooxygenase-2 (Cox-2) expression and myeloperoxidase activity in the lung are also decreased.
Conclusions: Transient RelB over-expression decreases the pro-inflammatory effects of long-term cigarette smoke exposure. Thus, RelB appears to function as a "brake" for dampening pre-existing inflammation in the lung. Increased expression and/or activation of RelB would be a novel therapeutic strategy against COPD and other inflammation-associated lung diseases.
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PS 2325 ADJUVANT EFFECT OF 1Ç3-β-GLUCAN (ZYMOSAN) EXPOSURE IN A MOUSE OVALBUMIN ALLERGY MODEL.

S. Young, M. Wolfarth, J. R. Roberts, M. L. Kashon and J. M. Antonini. *NIOSH, Morgantown, WV.*

A strong association has been observed between indoor mold contamination and the development of lung allergy and asthma. However, the relationship is still not fully understood. 1→3-β-Glucan is the major cell wall component of fungi, and has been shown to be a good marker of fungi exposure. The objective of the study was to evaluate the adjuvant effect of 1→3-β-glucan exposure during ovalbumin (OVA) sensitization in a mouse allergy model. BALB/c mice were sensitized by pharyngeal aspiration with saline (vehicle control), 50 μg OVA, or OVA with various doses of zymosan (1, 10, 50, or 75 μg) at days 0, 7, and 14. One week after sensitization, each sensitized animal group was challenged with aspiration dose of OVA 50 μg once a week for two weeks. At 24 hrs after the last aspiration, bronchoalveolar lavage (BAL) was done, and the following inflammatory and lung injury markers were measured in recovered BAL fluid: (1) leukocytes infiltration, (2) albumin and lactate dehydrogenase levels, and (3) inflammatory cytokines levels. An additional set of non-lavaged mice from each group was sent for lung pathology evaluation. The results indicated that co-exposure of zymosan and OVA during sensitization induced a synergistic inflammatory/allergic response that was greater than the response of the individual exposures when combined together. The optimum adjuvant dose of zymosan was 10 μg, inducing a significant eosinophil infiltration in the lungs and blood as analyzed by flow cytometry. This was consistent with the pathological findings of significant increases in perivascular lymphocyte, alveolar macrophage, neutrophil, and eosinophil infiltration when compared to the OVA-OVA group. Furthermore, a significant elevation in cytokines (IL-5, IL-13, IL-6, and TNF-α) in BAL fluid and increased OVA-specific IgE in blood were observed indicating an allergic inflammation. This study demonstrated an adjuvant effect of 1→3-β-glucan (zymosan) when exposed during the sensitization phase in an OVA-induced allergy model in BALB/c mice.

PS 2326 ROLE OF SURFACTANT PROTEIN-D (SP-D) IN REGULATING MACROPHAGE PHENOTYPE, INFLAMMATION, AND LUNG FUNCTION IN ELDERLY MICE FOLLOWING OZONE (O3) INHALATION.

A. M. Groves[1], J. D. Laskin[2], A. J. Gow[1] and D. L. Laskin[1]. [1]*Rutgers University, Piscataway, NJ and* [2]*UMDNJ-RWJ Medical School, Piscataway, NJ.*

SP-D is a pulmonary collectin important in controlling inflammatory responses. SP-D-/- mice develop progressive inflammation, characterized by accumulation of enlarged, activated macrophages (MP)s and an emphysematous morphology. Epidemiological data indicate that older individuals with emphysema are hypersensitive to O3. Mechanisms underlying this response were analyzed using SP-D-/- mice. O3 inhalation (0.8 ppm, 3 hr) resulted in increased MPs in BAL from younger (8 wk), but not older (27 wk) SP-D-/- mice 72 hr post exposure. Inflammation was also increased in tissue sections from younger SP-D-/- mice. In both younger and older mice, O3 effects on Newtonian resistance were greater in SP-D-/- mice relative to WT mice. O3 also increased lung compliance in younger SP-D-/- mice, but not younger WT mice. In contrast, in older mice, increases in compliance were only noted in WT mice. We next determined if these differential responses were correlated with MP phenotype. Activated MPs are classified into proinflammatory/cytotoxic M1 MPs, and anti-inflammatory/profibrotic M2 MPs. Immunohistochemical analysis of lungs from young WT mice showed low level MP expression of the M1 marker, inducible nitric oxide synthase (iNOS), and the M2 markers, arginase II (ArgII), YM-1 and mannose receptor (MR). Whereas in younger mice, loss of SP-D resulted in increases in M2 MPs, in older SP-D-/- mice both M1 and M2 MPs were seen. O3 inhalation caused a shift towards an M2 phenotype in younger and older WT mice, as assessed by increased ArgII, YM-1 and MR expression, with no change in iNOS. In contrast in SP-D-/- mice, O3 caused a shift towards an M1 MP phenotype, as evident by increased iNOS, with little change in ArgII, YM-1 or MR, and in older mice by decreased expression of MR. These results suggest that SP-D plays a key role in regulating MP phenotype, pulmonary inflammation and lung function in elderly mice following O3 inhalation (NIH ES004738, GM034310, CA132624, AR055073, HL074115, ES005022).

PS 2327 GLUTATHIONE DEPLETION POTENTIATES CIGARETTE SMOKE INDUCED AIRWAY INFLAMMATION.

B. J. Day[1, 2, 3], E. Min[1] and N. S. Gould[1, 2]. [1]*Medicine, National Jewish Health, Denver, Co.,* [2]*Pharmaceutical Sciences, University of Colorado AMC, Aurora, CO and* [3]*Medicine, University of Colorado AMC, Aurora, CO.*

Cigarette smoke (CS) is one of the main causes of respiratory disorders including emphysema and chronic bronchitis. A common underlying factor in these diseases is an exaggerated inflammatory response. CS causes an increase in inflammation and oxidantion which triggers an upregulation of glutathione (GSH). The role and effect of the upregulation of GSH on inflammatory pathways has not been completely established. Recent data shows that GSH levels found in the epithelial lining fluid (ELF) of the lung are inversely correlated to airway TNFα. Furthermore, macrophage activation and TNFα release is exaggerated in low GSH environments. The present study sought to determine if there was a direct link between GSH and inflammation that may be potentiated by CS. L-Buthionine-sulfoxime (BSO) in the drinking water was utilized as an inhibitor of GSH synthesis in vivo and mice were administered either normal or BSO water for 7 d pretreatment and then exposed to either air or CS for 5 d. Body weights were taken as an indicator of health throughout the experiment. During the pretreatment phase, body weights did not differ significantly between the groups, but during the CS exposure BSO administered mice lost roughly 10% of their body weight from peak levels while there was no significant change in other groups. An analysis of ELF GSH showed significant increases in the CS only mice while BSO blunted the increase with the BAL cell and lung tissue GSH showing similar trends while CS depleted GSH in the plasma and liver. Myeloperoxidase (MPO) activity, a marker of neutrophil infiltration, was three times higher in the lungs of BSO CS mice compared to BSO alone and TNFα levels were elevated in both BSO air and CS exposed mice. In contrast, air and CS only exposed mice did not have any increases in airway TNFα. These data suggest that GSH plays a role in CS induced inflammation which may help to explain why so many inflammatory diseases are associated with decreased GSH levels.

PS 2328 THE GLUTATHIONE SYNTHESIS GENE GCLM MODULATES PULMONARY INFLAMMATION FOLLOWING INTRANASAL INSTILLATION OF DIESEL EXHAUST PARTICULATE.

C. Weldy[1], C. C. White[1], D. P. Cox[1], H. Wilkerson[1], S. Gill[2], W. Parks[2] and T. J. Kavanagh[1]. [1]*Environmental and Occupational Health Sciences, University of Washington, Seattle, WA and* [2]*Pulmonary and Critical Care Medicine, University of Washington, Seattle, WA.*

Diesel exhaust particulate (DEP) is a major component of airborne particulate matter (PM) and exposure to DEP has been associated with pulmonary irritation, inflammation and exacerbation of asthma symptoms. DEP is a known pro-oxidant and can produce inflammation in the lungs by producing reactive oxygen and nitrogen species. Glutathione (GSH) is an important antioxidant thiol and the rate-limiting enzyme in the synthesis of GSH is glutamate cysteine ligase (Gcl), composed of catalytic (Gclc) and modifier (Gclm) subunits. As oxidative stress is believed to play a role in DEP-induced inflammation, we hypothesized that mice with compromised GSH synthesis (Gclm +/-, -/-) would be more susceptible to DEP-induced inflammation. In the present study, we dosed C57BL/6 WT, Gclm +/-, and Gclm -/- mice with DEP by intranasal instillation. Animals received a total of 20μl of PBS or 20μl of 10mg/ml DEP solution in one 10μl instillation per nostril. After 6 hours, animals were sacrificed and bronchial alveolar lavage fluid (BALF) was collected. Gclm +/- mice had increased neutrophilia and IL6 and TNFα in BALF than both WT and Gclm -/- mice. In contrast to this finding, analysis of lung tissue gene expression by qPCR revealed that Gclm -/- mice had elevated levels of mRNA transcripts for TNFα, IL6, GMCSF, IL1β, and MCP1 relative to both WT and Gclm +/- mice after DEP treatment. These data suggest that although Gclm -/- mice had a greater inflammatory gene expression response to DEP than WT or Gclm +/-, they may be compromised in their ability to subsequently synthesize or release inflammatory cytokines, and recruit neutrophils into the alveolar space. These results indicate that GSH is an important factor in regulating inflammatory response following DEP treatment. This work was supported by NIEHS grants 1P50ES015915, P30ES07033 and T32ES07032.

PS 2329 AIRBORNE ORGANIC PARTICULATE MATTER AS A LUNG INFLAMMATION INDUCER: ROLE OF PHOSPHOLIPASE A2.

S. R. Kotha[1], M. G. Piper[1], B. P. Caristophe[1], N. Patric[1], R. M. Uppu[2], M. B. Clay[1] and N. L. Parinandi[1]. [1]*Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, The Ohio Stae University College of Medicine, Columbus, OH and* [2]*Department of Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.*

Agricultural particulate matter (PM) has been linked to respiratory disorders including occupational asthma in the workers at Central Ohio poultry farms. In order to test the precise mechanism(s), human lung epithelial cells (A549), in culture, were treated with varying concentrations of poultry PM (0.1-1.0 mg) for different periods of time (0-60 min) following which Reactive oxygen species (ROS) production, PLA2 activity, cPLA2 serine phosphorylation, ERK1/2 phosphorylation (activation), and IL-8 release were determined. The results showed a significant activation of PLA2 induced by poultry PM (1.0 mg/mL) at 1 h, which was significantly attenuated by AACOCF3 (cPLA2 inhibitor) and PD98059 (ERK1/2 upstream inhibitor), suggesting the modulation of cPLA2 activity by ERK1/2 in cells. ERK1/2 phosphorylation was also induced by poultry PM which also paralleled cPLA2 serine phosphorylation. cPLA2 immunoprecipitates from poultry PM-treated cells exhibited enhanced association of ERK1/2 and cPLA2 in a time-dependent fashion. ERK1/2 inhibition with PD98059 also attenuated the poultry PM-induced serine phosphorylation of cPLA2 in cells. Furthermore, the poultry PM-induced cPLA2 serine phosphorylation and IL-8 release were significantly inhibited by AACOCF3 and PD98059, and by transfection with dominant-negative ERK1/2 DNA in cells. Finally, the poultry-induced IL-8 release by the bone marrow-derived macrophages of cPLA2 knockout mice was significantly lower. For the first time, this study demonstrated that the agricultural PM-induced IL-8 secretion by human lung epithelial cells was regulated by cPLA2 activation through ERK-mediated serine phosphorylation suggesting a probable mechanism of airway inflammation among poultry farm workers.

PS 2330 THE ROLE OF TRANSFORMING GROWTH FACTOR BETA 1 (TGF-β1) IN TOBACCO-SMOKE-INDUCED LUNG INJURIES.

L. L. Hoang and K. E. Pinkerton. *Center for Health and the Environment, University of California, Davis, Davis, CA.*

Rationale: Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States with 80-90% of all COPD cases attributed to smoking. Clinical studies have shown strong correlations between the progression of COPD and small airway remodeling indicated by increased small airway wall thickness, airway inflammation, mucus hypersecretion, and increased airway smooth muscle mass. However, the mechanism of small airway remodeling in COPD remains elusive. Previous clinical research demonstrated increased TGF-β1 expression in the lungs of COPD patients. TGF-β1 could be critical to small airway remodeling in COPD due to its ability to regulate both immune cells and airway structural cells. Therefore, we used TGF-β1+/- mice exposed to tobacco smoke to study the functions of TGF-β1 in tobacco smoke induced lung injury. Methods: TGF-β1+/- mice were exposed to tobacco smoke at 150 mg/m3 for 6 hours/day, 5 days/week for one month. Lungs were lavaged for analysis of cell viability, number and cell differential. Tracheas and lung lobes were frozen for qRT-PCR experiments. Results: Compared to wild type mice, TGF-β1+/- mice exhibited an increase in inflammatory cells recovered in the bronchoalveolar lavage fluid following smoke exposure. TGF-β1+/- mice exposed to tobacco smoke also showed a reduction in tracheal Muc5b mRNA expression. Smooth muscle alpha actin mRNA expression was also found to be elevated in the lungs of TGF-β1+/- mice following smoke exposure. Conclusions: We conclude TGF-β1 may protect the lungs from tobacco smoke induced pulmonary inflammation. TGF-β1 may also play a critical role to regulate mucus production and decrease airway smooth muscle hypertrophy during tobacco smoke exposure. Funded by: NIEHS (ES 011634 and ES013932) and TRDRP (18XT-0154)

PS 2331 RESPIRATORY AND SYSTEMIC RESPONSES TO URBAN SUMMER AND WINTER SUB-MICRON FINE AND ULTRAFINE PARTICULATE MATTER.

L. E. Plummer[1], C. M. Carosino[1], K. J. Bein[2], A. S. Wexler[2] and K. E. Pinkerton[1]. [1]*Center for Health and the Environment, University of California, Davis, CA and* [2]*Civil and Environmental Engineering, University of California, Davis, CA.*

Understanding seasonal impacts on adverse respiratory and systemic health effects can lead to more effective targeting of sources for improved control of particulate matter (PM) pollution, a concern for California's San Joaquin Valley (SJV). Toxicological evaluation of PM is critical in providing biological relevance for observed effects. Therefore, we tested the pro-inflammatory potential of SJV summer and winter PM sub-micron fine (SMF) and ultrafine (UF) from urban Fresno, CA in the lungs and blood of healthy male Balb/C mice. Inflammatory cell profile and cytotoxicity were measured 24 hours after intratracheal instillation of 25, 50 and 100 μg of PM/mouse. PM size distributions and oxidative potential were characterized using dynamic light scattering (DLS) and the cell-free dithiothreitol (DTT) assay, respectively. For all doses and both size-fractions, winter PM induced significantly greater acute pulmonary inflammation than equivalent doses of summer PM and was characterized by a robust, dose-dependent total leukocyte, neutrophil and lymphocyte influx. On an equal mass basis, winter SMF PM was more pro-inflammatory than winter UF PM however this difference was not statistically significant. Dose-dependent increases in circulating inflammatory cells and hematologic changes did not attain statistical significance. Particle measurements confirmed efficient dispersion and higher oxidative potential of winter versus summer PM for both size fractions. Pro-inflammatory potential of Fresno PM was significantly influenced by size and season. The relative toxicity within the lung was ranked: winter SMF > winter UF > summer SMF > summer UF. The potency of winter PM may be associated with observed DTT activity of winter SMF and UF PM. These studies are the initial results for source-oriented particle sampling and toxicity testing designed to elucidate key source contributors to PM toxicity. Funded by CARB/EPRI.

PS 2332 PARTICLE UPTAKE OF GASEOUS AIR TOXICS MODIFIES OBSERVED TOXICITY OF PM.

S. M. Ebersviller[1], K. Lichtveld[1], J. Zavala[1], Y. Lin[1], K. G. Sexton[1], I. Jaspers[1, 2] and H. Jeffries[1]. [1]*Environment Sciences . and Engineering, UNC-CH, Chapel Hill, NC and* [2]*CEMALB, UNC-CH, Chapel Hill, NC.*

The National Research Council has called for efforts toward determining whether 'effect modification' occurs between particulate matter (PM) and gases that surround it in the environment. Researchers have long known that gases and particles interact via dynamic processes in the atmosphere. What has been unclear to this point is if these interactions affect the actual toxicity of each phase. To investigate these processes, a series of experiments was conducted in an outdoor smog chamber capable of mimicking the ambient environment. In vitro cell cultures were exposed to the gas and particle phases of air mixtures using gas and particle exposure systems coupled directly to the chamber, thereby allowing exposures to occur without sampling artifacts. Mineral oil aerosol (MOA) acted as a non-toxic analog to ambient PM, as it possesses representative complexity in composition but lacks any inherent toxicity that may interfere with observations. A mixture of 55 compounds was injected into the chamber to represent a typical urban atmosphere. Exposures to the fresh chamber contents were performed both with and without MOA present. Chamber contents were allowed to react in natural sunlight for one day and exposures were repeated, again with and without MOA. As observed in previous work, the fresh mixture induced no response from exposed cells with either in vitro method, while there was a dramatic increase in response from cells exposed to the aged mixture using the gas-only system. For the system in which there was no PM present, there was no response from the cells exposed with the particle-only method. When MOA was added to the chamber after sundown, however, there was more than 6X the inflammatory response than was observed for any controls or the fresh exposure. As the MOA was added after sunset, the only source this increase in toxicity was the uptake of toxic species from the air surrounding the aerosol. These results together provide clear proof that 'effect modification' can, and is likely to, occur in the ambient environment.

PS 2333 CONTRIBUTIONS OF TRPV1 AND TRPM8 TO LUNG INJURY BY COAL FLY ASH.

C. A. Reilly, C. E. Deering-Rice, M. E. Johansen, J. K. Roberts, E. G. Romero, J. M. Veranth and G. S. Yost. *Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.*

Acute and long-term heath deficits are correlated with exposure to environmental particulate pollutants (PM). However, the molecular sensors that detect, differentiate, and respond to PM are often unknown. TRPV1 has been implicated as a sensor of PM and activation of TRPV1 has been correlated with inflammation, cell death, and altered cardiopulmonary function. This research investigated TRPV1 activation using a series of prototype PM, established a role for TRPV1 in pulmonary injury following instillation of PM into lungs of mice, and identified mechanisms of TRPV1 activation. A screen of TRPV1 activation by 8 prototype PM identified a coal fly ash PM (CFAm), silica, and diesel exhaust PM (DEP) as agonists. CFAm did not activate TRPA1, V3, or V4, but activated TRPM8. Like capsaicin, CFAm induced the expression of CXCL-1/KC, CXCL-2/MIP-2α and IL-6 in distal airways, and pro-apoptotic GADD153 in alveoli, consistent with lung injury in C57Bl/6 mice. CFAm activated cultured mouse DRG neurons and induced the expression of IL-6, IL-8, and GADD153 in primary human bronchial epithelial (NHBE) cells, all of which were attenuated by the TRPV1 antagonist LJO-328. Accordingly, CFAm-induced lung injury in TRPV1-/- mice was reduced. Calcium flux in TRPV1 over-expressing human bronchial epithelial cells by CFAm was inhibited by co-treatment by LJO-328 and by inhibition of cell surface TRPV1 using EGTA and ruthenium red. Responses were also exacerbated by enriching TRPV1 at the cell surface. TRPV1 activation was attributable to the solid core fraction of CFAm and occurred through an interaction with residues adjacent to the pore-loop segment of TRPV1. These data show an additive role for sensory nerve and non-neuronal TRPV1 in mediating lung injury by a model PM and reveal a novel mechanism of activation involving PM contact with cell surface residues that may also occur for other PM that activate TRPV1. Additionally, TRPM8 may also contribute to lung injury by this PM. Support: ES017431 and HL069813.

PS 2334 ACTIVATION OF HUMAN TRPA1 BY DIESEL EXHAUST PARTICLES (DEP): ASSOCIATION WITH LUNG INJURY.

C. Deering-Rice, E. G. Romero, J. M. Veranth, G. S. Yost and C. A. Reilly. *Pharmacology/Toxicology, University of Utah, Salt Lake City, UT.*

Inhalation exposure to environmental particulate air pollutants (PM) is correlated with a number of adverse health effects in humans. However, the molecular "sensors" that detect, differentiate, and initiate deleterious responses to PM that likely culminate as compromised health are not known. TRPV1 and TRPA1 orchestrate responses to a number of prototype respiratory toxicants including capsaicin, acrolein, and crotonaldehyde. The hypothesis of this study was that TRPA1, and not TRPV1, would be activated by specific forms of environmental PM in vitro and that activation in vivo would result in lung injury. A screen of 6 prototype PM revealed that diesel exhaust PM (DEP) activated TRPA1 and that bioactivity was retained in ethanol extracts. Ethanol extracts of DEP failed to activate TRPM8, V1, V3 and V4. The extract induced calcium flux in isolated mouse DRG neurons sensitive to the prototype TRPA1 agonist AITC and were abolished by co-treatment

with the TRPA1 antagonist HC-030031. Efforts are underway to establish the mechanism of TRPA1 activation by the DEP extract and to purify the individual chemicals responsible for TRPA1 activation. This study identified TRPA1 as a specific molecular sensor for components of DEP and suggest that activation of TRPA1 in airway sensory nerves may be the critical first-step in DEP toxicity in the respiratory tract. This work was supported by ES017431 and HL069813.

PS 2335 MECHANISMS OF CHLORINE HYPERSENSITIVITY IN ASTHMA.

S. Balakrishna[1], B. F. Bessac[1], W. Song[2], A. I. Caceres[1], T. Nicola[3], S. F. Doran[2], A. Yadav[2], N. Ambalavanan[3], S. Matalon[2] and S. E. Jordt[1]. *[1]Pharmacology, Yale School of Medicine, New Haven, CT, [2]Anesthesiology, University of Alabama at Birmingham, Birmingham, AL and [3]Pediatrics, University of Alabama at Birmingham, Birmingham, AL.*

Asthma remains one of the most common lung diseases, afflicting nearly 22.9 million Americans, including approximately 6.8 million children. Any short-term high-level exposures to pulmonary agents or industrial irritants, including chlorine due an unsolicited industrial accident or a catastrophe can pose a high threat to the asthmatic populace. In asthmatics even low levels of chlorine exposure can exacerbate airway inflammation, and can induce rapid airway constriction and obstruction, bronchospasm, mucus secretion and pain. Our preceding studies affirm that the ion channel TRPA1 as the primary chlorine sensor in airway sensory neurons, mediating respiratory sequelae that may include increased airways reactivity and inflammation. In asthmatics, these neurons are highly sensitized to chemical stimuli. We hypothesize that post-exposure administration of TRP channel antagonists, in combination with adrenergic agonists and antioxidants; will counteract life-threatening hypersensitivity responses in asthmatics to chlorine.
Experimentally induced asthmatic animals were exposed to high or low levels of chlorine and lung mechanics were measured after 24 h post chlorine exposure. BALF, serum, lungs were isolated and analyzed for various inflammation and injury markers. Our findings demonstrated that both high-level and low-level Chlorine exposures cause strong increases in airway resistance and inflammation in asthmatic mice. Robust biomarkers (IL-4 and MPO) for asthmatic inflammation aggravated by chlorine exposures were identified. We conclude that chlorine-exposed asthmatics represent a high risk patient prone to higher levels of injury and inflammation. Asthmatic bystanders exposed to low chlorine levels may develop strong asthmatic responses hours or days following exposure.

PS 2336 BLEOMYCIN-INDUCED LUNG ENDOTHELIAL CYTOSKELETAL REARRANGEMENT IS REGULATED BY THIOL-REDOX.

R. B. Patel[1], L. Sauers[1], R. M. Uppu[2] and N. L. Parinandi[1]. *[1]Division of Pulmonary, Allergy, Critical Care, and Sleep Medicine, The Ohio State University College of Medicine, Columbus, OH and [2]Department of Environmental Toxicology, Southern University and A&M College, Baton Rouge, LA.*

As lung vascular alterations and pulmonary hypertension associated with oxidative stress and thiol-redox alterations have been reported to be involved in the idiopathic lung fibrosis (ILF), here, we hypothesize that the lung fibrosis inducer, bleomycin, causes cytoskeletal rearrangement through thiol-redox alterations in the cultured lung vascular endothelial cell monolayers. We exposed the monolayers of primary bovine pulmonary artery and microvascular endothelial cells to bleomycin (5 and 10 μUnits) and studied the cytotoxicity, cytoskeletal rearrangements, and the macromolecule (FITC-dextran, 70,000 Mol.Wt) paracellular transport in absence and presence of the thiol-redox protectants. Our results revealed that bleomycin induced cytotoxicity (LDH leak), morphological alterations (rounding of cells and filipodia formation), and cytoskeletal rearrangement (Actin stress fiber formation and alterations of tight junction proteins, ZO-1 and occludin) in a dose-dependant fashion. Moreover, our study demonstrated the formation of reactive oxygen species, loss of thiols (GSH), endothelial barrier dysfunction (decrease of trans-endothelial electrical resistance) and enhanced paracellular transport (leak) of macromolecules (FITC-Dextran). All these bleomycin-induced alterations in ECs were attenuated by the thiol-redox stabilizer, N-acetylcysteine (NAC), suggesting the central role of thiols in vascular endothelial dysfunction associated with ILF.

PS 2337 HMGB1 IN HYPEROXIA-COMPROMISED HOST ABILITY TO CLEAR *PSEUDOMONAS AERUGINOSA* PNEUMONIA.

V. S. Patel[1], R. Sitapara[1], B. Phan[1], A. Gore[1], R. Malla[1] and L. L. Mantell[1, 2]. *[1]Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY and [2]The Feinstein Institute for Medical Research, North Shore-LIJ Health Science, Manhasset, NY.*

Mechanical ventilation with supraphysiological concentrations of oxygen (hyperoxia) is routinely used to treat patients with respiratory distress. However, patients on ventilators have increased incidence of inflammatory lung injury and enhanced susceptibility to bacterial infection. *Pseudomonas aeruginosa* is one of the most common bacteria found in patients with ventilator-associated pneumonia. Our previous studies demonstrate that hyperoxia compromises macrophage's ability to phagocytose bacteria and HMGB1 can directly cause macrophage dysfunction. The objective of this study was to elucidate the role of HMGB1 in *Pseudomonas aeruginosa* pneumonia under hyperoxic conditions. In this study, we show that pre-exposure to hyperoxia (≥99.0% O_2) resulted in an increased lethality in C57BL/6 mice infected with *Pseudomonas aeruginosa*. Accompanied with the increased mortality, levels of airway HMGB1 were elevated in these animals. Treatment of these mice with neutralizing anti-HMGB1 monoclonal antibody attenuated lung injury, and reduced bacterial counts in the lung and the levels of airway HMBG1. These results indicate that HMGB1 plays a critical role in hyperoxia-compromised host ability to clear bacteria, suggesting that HMGB1 may provide a novel target in the treatment of ventilator-associated pneumonia.

PS 2338 A WHOLE BODY INHALATION EXPOSURE SYSTEM FOR THE OIL DISPERSANT COREXIT 9500 WITH PULMONARY FUNCTION RESULTS FROM AN INITIAL SET OF EXPOSURES WITH RATS.

W. T. Goldsmith, W. G. McKinney, M. C. Jackson, J. S. Reynolds, J. Cumpston and D. G. Frazer. *HELD, CDC/NIOSH, Morgantown, WV.* Sponsor: A. Hubbs.

In response to the Deepwater Horizon oil spill, millions of gallons of chemical dispersant (mainly COREXIT 9500) were sprayed or pumped into the oil to accelerate the dispersal process. To examine the health effects of inhaled dispersants, an automated whole body inhalation exposure system was designed. COREXIT 9500 was placed in a glass syringe. Since the dispersant settled over time, a Teflon magnetic stir bar was also positioned in the syringe and a spinning magnet was employed to continually mix the liquid. A syringe pump injected the dispersant into an atomizer where droplets were formed and mixed with clean, dry air pressurized at 35 PSI. The aerosol was mixed with diluent air that was regulated with a mass flow controller (MFC). The diluted aerosol then was passed into a custom stainless steel and acrylic exposure chamber with 12 individually housed rats. Exhaust air was HEPA filtered and then run through a MFC attached to a vacuum source. A pressure transducer was used to monitor chamber pressure. A light scattering instrument (LSI) estimated the real-time particle mass concentration of the aerosol in the chamber. Gravimetric samples were used to calibrate the LSI. A custom software GUI was developed to record and control parameters during the exposures. Software feedback loops controlled the chamber aerosol concentration and pressure during exposures. Particle size of the dispersant aerosol was measured and shown to have a number peak at 271 nm and a mass peak at 961 nm. Chamber concentration homogeneity was established by placing multiple gravimetric samplers in various parts of the chamber during the same time period. Three individual exposures were conducted in which Sprague Dawley rats were exposed to 15 mg/m^3 of COREXIT 9500 for 5 hours. Breathing rate and specific airway resistance comparisons between exposed (n=36) and control (n=36) animals showed no significant change immediately post, 1 day post and 7 days post exposure.

PS 2339 GENE AND PROTEIN EXPRESSION BIOMARKERS IN FUNGICIDE EXPOSED ZEBRAFISH.

D. C. Bencic[1], R. Wang[1], A. D. Biales[1], R. W. Flick[1], D. L. Villeneuve[2], G. T. Ankley[2] and D. L. Lattier[1]. *[1]U.S. EPA/ORD/NERL/EERD, Cincinnati, OH and [2]U.S. EPA/ORD/NHEERL/Med., Duluth, MN.* Sponsor: H. Choudhury.

The research presented here is part of a large, integrated project attempting to 1) identify new molecular biomarkers of exposure to endocrine disrupting compounds (EDCs) in model small fish species and 2) link these biomarkers to effects that are relevant for both diagnostic and predictive risk assessments. The chemicals represent several different modes of action (MOA) with known or hypothesized impacts on the hypothalamic-pituitary-gonadal (HPG) axis. In this study, the impact of prochloraz (PCZ) on reproductively mature male and female zebrafish was exam-

ined following up to 96 h continuous exposure in a flow-through system to control (water only), low (100 μg/l), and high (500 μg/l) PCZ doses. An imidazole fungicide used to protect food crops by weakening fungal cell membranes through CYP51 inhibition, PCZ is known to have mixed MOA in vertebrates. Prochloraz functions as an aromatase (CYP19) inhibitor as well as an androgen receptor antagonist. Total RNA was isolated from female brains and male and female gonads after 48 and 96 h exposures. Gene expression changes were examined using microarrays (n=5 per sex/dose/time). Tissue- and sex- specific gene expression differences ($p<0.05$; FDR<5%) were observed, typically 150-300 differentially-expressed (> 1.3 fold change) genes per treatment. Protein expression changes in male and female brains were examined using two-dimensional gel electrophoresis. Approximately 25 differentially expressed proteins were identified in each gender from a total population of 1300-1400 proteins. About 70% of the proteins in each sex were up-regulated and two were common between the sexes. These differential expression profiles will be used to help identify new molecular biomarkers of exposure and integration of these data with whole organism endpoints will provide a more definitive description of the mixed MOA of PCZ. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

PS 2340 GENES FROM BONE MARROW OF BENZENE TREATED MICE, COUPLED WITH STRUCTURAL ANALYSIS OF BENZENE AND SIMILAR CHEMICALS IN OIL SPILLS, JOINTLY PREDICT ACUTE MYELOID LEUKEMIA AND POSSIBLE MECHANISM.

D. Mitic Potkrajac[1], G. Apic[1], F. Bonner[1] and R. B. Russell[2]. [1]*Cambridge Cell Networks Ltd., Cambridge, United Kingdom and* [2]*Cell Networks, University of Heidelberg, Heidelberg, Germany.* Sponsor: P. Hewitt.

The aim of our study was to predict target organ toxicity of benzene and other compounds present in oil spills by combining two methods in a systematic way: chemical structure analysis and gene expression analysis from mouse bone marrow. Additionally, we applied an in silico systems biology approaches to generate hypotheses on mechanism of action for these chemicals. Benzene is an established hematotoxicant and leukemogen, with its reactive metabolites inducing cyto- and genotoxicities. We analyzed expression data of bone marrow from 7-week male C57BL/6 mice, exposed to 300ppm benzene for 6h/day, 5 days/week for 2 weeks (1). From the whole genome analysis, 69 signature genes were selected as significantly dysregulated For these genes our approach, which uses a network of previously determined genes, proteins, chemicals and toxicities, predicted and ranked several hundred toxicities, with leukemias being most strongly indicated. The prediction for Acute myeloid leukemia (AML) included 17 of 69 genes. When combined with benzene and its cellular metabolites, the mechanism of AML implicates roles of genes involved in DNA damage response, cell cycle regulation, apoptosis and hemopoiesis. We also used known molecular interactions between chemicals similar to benzene and genes and proteins in our vast information network. Relationships with pathologies mentioned above suggested a mechanistic flow of how the compounds lead to the pathologies. This study shows that the incorporation of indirect evidence (ie. chemical-gene relations) can suggest a mechanistic picture for more genes than traditional analysis and shows how simultaneous exposure to similar chemicals can affect gene expression and the observed toxicity. 1. Yoon B-IL et al, 2003. Mechanism of Benzene-Induced Hematotoxicity and Leukemogenicity: cDNA Microarray Analyses Using Mouse Bone Marrow Tissue Environmental Health Perspectives 2003. Aug;111(11):1411-20

PS 2341 CHEMICAL DISPERSANTS USED IN THE GULF OF MEXICO ARE CYTOTOXIC AND GENOTOXIC TO HUMAN SKIN FIBROBLASTS.

J. Wise[1, 2], S. Wise[1, 2, 3], H. Xie[1, 2, 3] and J. P. Wise[1, 2, 3]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME,* [2]*Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME and* [3]*Department of Applied Medical Science, University of Southern Maine, Portland, ME.*

On April 20th, 2010, the Deepwater Horizon Oil Rig exploded in the Gulf of Mexico, causing millions of barrels of oil to gush into the ocean. One effort to contain the oil was to apply chemical dispersants to break up the oil. However the toxicity of these dispersants is unknown. The objective of this study is to determine the toxicity of the two dispersants (EC9500A and EC9527A) used in the Gulf. Specifically, we determined the genotoxicity and cytotoxicity of the dispersants in human skin (BJhTERT) and lung (WHTBF-6) fibroblasts. Cells were treated with and without S9 fractions with cofactors, because fibroblast cells do not readily express p450 enzymes necessary to metabolize the chemicals. The EC9500A data show that dispersants are cytotoxic to skin cells. Specifically, 1, 5 and 10 percent EC9500A induced 89, 108 and 9 percent relative survival, respectively. S9-mediated metabolism increased toxicity inducing 77, 82 and 0 percent relative survival, respectively. It was also genotoxic. S9-medtiated metabolism was required for genotoxicity. Specifically, in the presence of the S9 fraction, 1 or 5 percent EC9500A induced damage, in 0 and 5 percent of metaphase cells, producing 0 and 12 total aberrations per 100 metaphases, respectively. 10 percent EC9500A caused cell cycle arrest. Data for human lung cells are pending. This work is supported by the Maine Center for Toxicology and Environmental Health.

PS 2342 DEVELOPING A WHALE CELL LINE AT SEA TO EVALUATE THE CYTOTOXICITY AND GENOTOXICITY OF CHEMICAL DISPERSANTS USED IN THE GULF OF MEXICO OIL CRISIS.

C. F. Wise[1, 2, 4], M. Braun[1, 2, 4], J. P. Wise[1, 2, 4], S. S. Wise[1, 2, 3], I. Kerr[2, 4], H. Xie[1, 2, 3] and J. P. Wise[1, 2, 3]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME,* [2]*Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME,* [3]*Department of Applied Medical Science, University of Southern Maine, Portland, ME and* [4]*Ocean Alliance, Lincoln, MA.*

In April 2010, the Deepwater Horizon Oil Rig exploded causing millions of gallons of crude oil to gush into the Gulf of Mexico. This catastrophe resulted in the most disastrous offshore oil spill that the United States has ever experienced. Humans depend on the Gulf as well as many animal species that reside in it or use the area as their spawning or calving grounds. As a result of the crisis, these animals were exposed to copious amounts of crude oil and exceptional amounts of chemical dispersants that were sprayed on the oil in an attempt to clean up the spill. There have been few studies done to understand the toxicological effects of the chemical dispersants themselves or the combination of oil and dispersant. One key species in the Gulf are whales. These animals are good indicators for both human and ocean health. They are mammals that breathe air and are at the top of the food chain. Our hypothesis is that chemical dispersants are cytotoxic and genotoxic to whale cells. However, to test this hypothesis, it was essential to have a whale specific model to study. Whales are not laboratory animals, and thus, a cell culture model was needed. Accordingly, we installed cell culture laboratory on a boat, the first of its kind at sea. Whale skin biopsies were collected at sea with a biopsy dart delivered with a crossbow. Whale skin fibroblasts were grown from explants of the interface between the blubber and skin. Once these primary cells were expanded into a primary cell line, they were treated with dispersants and the cytotoxicity and genotoxicity determined with and without an S9 fraction. In order to contextualize cell culture data, we also measured dispersant levels in the blubber of the animals.

PS 2343 BONE HOMEOSTASIS IS NOT ALTERED IN MCHR1-DEFICIENT MICE.

B. Gemzik[1], J. A. Tamasi[2], J. J. Devenny[2], B. J. Murphy[2], W. N. Washburn[3] and B. D. Car[1]. [1]*Discovery Toxicology, Bristol-Myers Squibb Company, Princeton, NJ,* [2]*Discovery Biology, Bristol-Myers Squibb Company, Princeton, NJ and* [3]*Discovery Chemistry, Bristol-Myers Squibb Company, Princeton, NJ.*

MCH (melanin concentrating hormone) is a peptide considered to play an important role in hypothalamic function in mammalian species. Selective antagonists of the MCHR1 receptor have been reported to reduce food intake and body weight gain, and produce anxiolytic and antidepressant effects in animal models. MCH is also speculated to be involved in hypothalamic regulation of bone mass, with an initial report of a reduction in cortical bone mineral density (osteopenia) in MCHR1-deficient mice. The objective of this study was to further characterize bone homeostasis in MCHR1-deficient mice. Mice of mixed strain, C57Bl/6 and Lex129SVEV, were bred with C57Bl/6 mice to provide age-matched (16 weeks) males and females of wildtype, heterozygous and homozygous knockout genotypes. Tibial and/or femur bone mineral density (trabecular and cortical), cortical microarchitecture, histology, and serum markers of bone resorption (tartrate-resistant acid phosphatase [TRAP] isoform 5b) and formation (osteocalcin) were evaluated as indices of bone homeostasis. There were no alterations in any of these parameters consistent with osteopenia or an effect on bone turnover in homozygous or heterozygous knockout mice compared to wild-type controls. In addition, serum TRAP 5b and osteocalcin, and bone histology were not altered in adult male rats given repeated doses of a selective small molecule MCHR1 antagonist. Together, these results indicate that normal bone homeostasis is maintained in the absence of MCHR1 signaling in rodents, and suggest that related hypothalamic pathways are not functionally obligate for maintenance of bone mass.

PS 2344 A NOVEL SENSITIVE AND SELECTIVE REAL-TIME CELLULAR ASSAY FOR DETECTION OF ENDOCRINE DISRUPTORS USING NATIVE ENDOCRINE SIGNALING PATHWAYS.

C. Jin[1], Y. Abassi[1], X. Xu[1], X. Wang[1], W. Zhang[2] and S. Gabos[2]. [1]*Acea Biosciences, San Diego, CA and* [2]*Alberta Health and Wellness, Edmonton, AB, Canada.* Sponsor: M. Lo.

A cell based assay for detection of endocrine disruptors was developed using real time impedance monitoring of native endocrine signaling pathways in T47D breast cancer cell line. T47D cells express estrogen receptor (ER) as well as some other steroid hormone receptors such as progesterone receptor (PR) and glucocorticoid receptors (GR). Stimulation of T47D cells with respective endocrine agonists leads to an increase in cell number which can be detected by gold microelectrodes embedded in the bottom of the well of specialized microelectronic plates. Addition of estrogen agonists including 17beta-estradiol, DPN and PPT to T47D cells induce a signature biphasic and dose-dependent increase in impedance readout. The observed EC50 values are comparable or better than existing in vitro cell based reporter assay systems. The specificity of the assay for ER activity was established using well characterized ER antagonists, such as ICI 182780, Raloxifene and ZK164015. Using this assay system, several environmental hazardous compounds known to disrupt endocrine signaling pathways were tested, including bisphenol A, daidzein, nonylphenol, and PBDE-47. All of these compounds induced a dose-dependent impedance-based response profile similar to ER agonists and were inhibited by ER antagonists. The calculated EC50 values were comparable to lowest published EC50 values generated in different assay systems. Moreover, the T47D system was also used to detect response to PR agonist, progesterone and GR agonist, dexamethasone. These two compounds also produced impedance-based biphasic responses although with different kinetic profile compared to ER activation. The data suggests the T47D assay system has the capacity to sensitively, selectively and quantitatively detect three different nuclear hormone responses.

PS 2345 THE IMPACT OF EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITION ON ENERGY HOMEOSTASIS.

M. Biggs[1], D. Threadgill[2], T. Ghashghaei[3] and T. Lee[4]. [1]*Toxicology, University of North Carolina, Chapel Hill, NC,* [2]*Genetics, North Carolina State University, Raleigh, NC,* [3]*Department of Molecular and Biomedical Sciences, North Carolina State University, Raleigh, NC and* [4]*Genetics, University of North Carolina, Chapel Hill, NC.*

As a result of the worldwide rise in obesity and obesity-related complications, such as diabetes, stroke, and cardiovascular disease, understanding the mechanisms associated with this disease is necessary. Obesity results from taking in more calories than required for energy homeostasis. Body weight is regulated by this balance between food intake and energy expenditure, through a highly integrated, multi-organ system. Accumulating evidence suggests signaling through the epidermal growth factor receptor (EGFR) is required for normal adipocyte development and, therefore, this signaling may be important in obesity development. Our lab has previously shown that EGFR inhibition retards adipose deposition in diet-induced obesity models, either pharmacologically with a small molecule inhibitor of the EGFR tyrosine kinase, AG1478, or genetically in a constitutionally impaired EGFR tyrosine kinase model ($Egfr^{wa2}$). However, the mechanisms behind this phenotype are unknown. Here we show that central EGFR signaling is important in appetite and metabolism regulation in diet-induced obesity. We found significantly less body weight and fat mass in mice with a conditional deletion of EGFR in the central nervous system fed a high-fat diet. Contributing to this decrease in fat mass is an increase in energy expenditure due to hyperphagia, increased oxygen consumption, diet-induced thermogenesis, and lipolysis in the white adipose tissue of these animals compared to control littermates. These findings suggest that altered signaling between the CNS and periphery, due to this increase in energy expenditure, accounts for this resistance to diet-induced obesity in mice with central EGFR inhibition.

PS 2346 SCIENTIFICALLY RELEVANT INFORMATION TO EVALUATE THE ENDOCRINE DISRUPTING POTENTIAL OF ISOPHORONE.

N. M. Berdasco and D. R. Geter. *Toxicology and Environmental Research and Consulting, The Dow Chemical Company, Midland, MI.*

In 1996, Congress directed the U.S. EPA to develop a prioritization and screening program to evaluate potential endocrine disrupting compounds. This program is now referred to as the Endocrine Disruptor Screening Program (EDSP). Tier 1 EDSP assays are composed of 5 in-vitro, 4 mammalian in vivo, and 2 eco-toxicity screening assays designed to identify substances with the potential to interact with the estrogen, androgen, or thyroid (EAT) hormone systems. Isophorone, a solvent and crop protecting compound, was selected in the initial round of EDSP testing. As part of the testing program, Other Scientifically Relevant Information (OSRI) for isophorone was presented to the EPA in consideration for aiding in the understanding of possible interactions with the EAT hormone systems. The isophorone OSRI included summarized information on a developmental toxicity study in rats, a limited one-generation reproduction toxicity study, 2 sub-chronic toxicity studies, and 2 chronic toxicity/oncogenicity studies. Isophorone was also assessed in two in vitro assays, an estrogen-receptor alpha (ERα) binding and an ERα transcriptional activation assay. In summary, this OSRI concluded that the solvent isophorone does not bind to either the estrogen or androgen receptor, was neither embryotoxic nor teratogenic, and did not alter pregnancy rates or litter sizes. From these data, it was concluded that isophorone did not demonstrate any pattern of effects consistent with adverse endocrine-mediated effects on reproductive organs or processes, nor were any alterations seen that support the potential for operating by an endocrine-related mode of action.

PS 2347 EXENATIDE AND SITAGLIPTIN: THEIR ROLE IN AUTOPHAGOSOME REGULATED TOXICITY IN PANCREATITIS.

R. Rouse, L. Zhang and D. A. Volpe. *U.S. FDA, Silver Spring, MD.*

The anti-diabetic drugs exenatide (EXN), a glucagon-like peptide 1 (GLP-1) receptor agonist, and sitagliptin (SIT), an inhibitor of dipeptidyl peptidase-4 (GLP-1 inhibitor) have been linked to post-marketing reports of drug-induced acute pancreatitis (DIAP). To better understand the risk of this effect, a series of in vitro studies were undertaken to investigate the toxicity mechanisms contributing to DIAP. MTS viability assays were performed to evaluate the proliferation of rat pancreatic acinar cells (AR42J), ductal cells (DSL-6A/C1) and islet cells (RIN-m5F) dosed with EXN or SIT for 8, 24 or 48 hrs. SIT was toxic in all cell types at 1 mg/ml at 8, 24, or 48 hrs. No viability decreases were found at concentrations below 0.1 mg/ml. At 48 hrs, 1mg/ml EXN induced proliferation in the islet cell line but inhibited ductal and acinar cell line proliferation. LC3 (autophagosome) co-localization with intracellular compartments marked with amylase (zymogen), LAMP2 or LAMP1 (lysosome), Rab7 (lysosome or late endosome), EEA1 (early endosome), and cathepsin B (activate trypsinogen) were also investigated using AR42J or primary rat acinar cells. The results obtained demonstrated effects of EXN and SIT on co-localization of autophagosomes in lysosomes and other intracellular compartments. Finally, we inhibited the activities or expressions of cathepsin B, trypsinogen and protease-activated receptor-2 (PAR-2) to evaluate the roles these proteins or pathways play in intracellular autophagosome localization. The inhibition of the above proteins altered the colocalization between autophagosome and other intracellular compartments induced by EXN or SIT. These results suggest that EXN and SIT may play a role in the onset of acute pancreatitis by altering vacuole accumulation and localization in acinar cells through several signaling pathways encountered in pancreatic cells.

PS 2348 IMPROVING THE *IN VIVO* PREDICTIVE VALUE OF THE ER-CALUX BIOASSAY BY COMBINING *IN VITRO* ESTROGENICITY RESULTS WITH KINETIC CHARACTERISTICS OF ESTROGENIC COMPOUNDS.

W. Brand[1], A. Punt[1, 2], A. J. Murk[2], M. Schriks[1], A. P. van Wezel[1] and M. B. Heringa[1]. [1]*KWR Watercycle Research Institute, Nieuwegein, Netherlands and* [2]*Division of Toxicology, Wageningen University, Wageningen, Netherlands.*

In vitro bioassays play an important role in effect-directed analysis, providing a rapid way of screening samples for the presence of bioactive compounds. An example is the ER-Calux bioassay, a reporter gene assay based on T47D cells stably transfected with an estrogen-responsive luciferase reporter gene, which allows sensitive detection of estrogenic (and anti-estrogenic) compounds. A limitation of many *in vitro* bioassays however, is that they generally do not take the ADME characteristics of compounds into account, which can hamper the *in vivo* predictive value of these assays.

The present study compares the estrogenicity of a suite of (xeno-)estrogenic compounds, relative to ethinyl estradiol (EE2) as determined with the ER-Calux bioassay, to literature-derived in vivo estrogenic responses obtained with the rat uteroptrophic assay. To correct for compound specific differences in ADME characteristics, albumin binding constants were determined for the suite of compounds, as well as hepatic availability (*i.e.* the dose that escapes hepatic metabolism), determined by incubations with liver microsomes and cytosol. The results demonstrate that combining the EC_{50} values for estrogenic activity determined with the ER-Calux bioassay with the compound-specific kinetic characteristics for albumin binding and hepatic availability, improves the correlation with the *in vivo*

effect doses obtained with the rat uterotrophic assay. As a result, a 2-fold more accurate confidence interval for the curve describing the EE2 equivalent dose - response curve was obtained. The present methodology increases the *in vivo* predictive value of the ER-Calux bioassay for the selected compounds, and could provide a method of deriving an *in vitro* effect-based benchmark dose needed for risk assessment of samples containing estrogenic compounds.

PS 2349 PCB-153 AND BDE-47 INCREASE THYROXINE (T_4) CATABOLISM IN RAT AND HUMAN HEPATOCYTES.

V. M. Richardson[1, 2] and M. J. DeVito[3]. [1]*ORD/NHEERL/ISTD, U.S. EPA, Research Triangle Park, NC,* [2]*Curriculum in Toxicology, UNC at Chapel Hill, Chapel Hill, NC and* [3]*Toxicology Branch, NIEHS/NTP, Research Triangle Park, NC .*

Previous studies demonstrate that *in vivo* exposure to 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) and 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) decrease serum thyroxine (T_4) levels in rats. This decrease is thought to occur through the induction of hepatic metabolizing enzymes resulting in the enhanced catabolism of T_4. There is some evidence the T_4 decrease occurs in humans, but the mechanism is unclear. Using primary rat and human hepatocytes, we compared the effects of PCB-153 and BDE-47 on T_4 catabolism. 48 hours after plating, fresh sandwich-cultured human or Sprague-Dawley rat hepatocytes were treated with PCB-153 or BDE-47 at a concentration of 30uM in 0.1% DMSO. 72 hours after dosing, cell culture media was replaced with media containing [I^{125}]-T_4 at median serum concentrations observed in rats and humans; 0.05uM and 0.1uM, respectively. 24 hours after [I^{125}]-T_4 administration, media was collected, T_4 and its metabolites were separated by UPLC, and fractions collected and quantified on a gamma counter. The predominant metabolite found in media of control and treated hepatocytes was T_4-glucuronide (T_4G). Results show a higher basal activity of T_4G formation in unexposed rat hepatocytes as compared to unexposed human hepatocytes (5.2 vs.0.1 fmol T_4G/min/mg protein). Following PCB-153 treatment, T_4G formation in rat hepatocytes increased 3.8-fold while increasing 10.8-fold in human hepatocytes. BDE-47 increased T_4G formation in rat and human hepatocytes by 1.6- and 13.3-fold, respectively. The data demonstrates that basal activity for T_4G formation in rat hepatocytes is approximately 50-times higher than human hepatocytes. Additionally, PCB-153 and BDE-47 increase T_4G formation in rat and human hepatocytes, with greater increases in humans. As a possible *in vitro* screening tool for environmental toxicants, the results highlight the utility of primary hepatocytes in evaluating potential species differences and human health risk.(This abstract of a proposed presentation, does not reflect USEPA or NIH policy.)

PS 2350 TRICLOSAN DECREASES RAT THYROXINE: MODE-OF-ACTION, DEVELOPMENTAL SUSCEPTIBILITY AND HUMAN RELEVANCE.

K. B. Paul[1, 2], J. M. Hedge[2], S. O. Simmons[2], M. J. DeVito[3, 1] and K. M. Crofton[2, 1]. [1]*Toxicology, University of North Carolina, Chapel Hill, NC,* [2]*Integrated Systems Toxicology Division, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC and* [3]*NTP, NIEHS, Research Triangle Park, NC .*

Triclosan (TCS) decreases rat serum thyroxine (T4). *In vivo* and *in vitro* approaches were used to address three uncertainties: by what mode-of-action (MOA) does TCS decrease T4; does TCS decrease T4 developmentally; and, are effects observed in rats relevant to humans? To test the hypothesized MOA that TCS decreases T4 via activation of the pregnane X and constitutive androstane receptors (PXR, CAR), and subsequent up-regulation of hepatic catabolism of T4, weanling female Long-Evans rats received TCS po (0-1000 mg/kg/day) for 4 days. Pentoxyresorufin-O-deethylase (PROD) and uridine diphosphate glucuronyltransferase (UGT) enzyme activities were measured in liver microsomes. qRT-PCR was used to measure mRNA expression of CYPs, UGTs, and sulfotransferases. PROD and UGT activities increased 8- and 2-fold at 1000 mg/kg/day. *Cyp2b2, Cyp3a1, Ugt1a1* and *Sult1c1* mRNA expression levels were induced 2- to 4-fold at 300 mg/kg/day. To test this MOA in dams and offspring, pregnant Long-Evans rats received TCS po (0-300 mg/kg/day) from gestational day (GD) 6 to postnatal day (PND) 21. Serum T4 decreased 30% in GD20 dams and fetuses, PND4 pups and PND22 dams at 300 mg/kg/day. Hepatic PROD activity increased 3-fold in PND22 dams and PND4 pups, and UGT activity was 1.5-fold in PND22 dams at 300 mg/kg/day. Reductions of 30% in T4 for dams and GD20 and PND4 offspring with concomitant increases in PROD and UGT activity suggest TCS may reduce T4 during development by a similar MOA to weanlings. To assess human relevance of the MOA, cell lines (Puracyp, Inc.) containing a CYP3A promoter-luciferase gene fusion were used to test activation of PXR in luciferase-based reporter assays. TCS moderately activated human PXR, but did not activate rat PXR. The data suggest that the initial key event of the MOA may be different between rats and humans, but the downstream effects may be similar between species. *This abstract does not necessarily reflect the policy of the US EPA or NIH.*

PS 2351 EVALUATION OF SUBSTANCES ROUTINELY TAKEN IN EVERYDAY LIFE IN THE H295R STEROIDOGENESIS ASSAY.

H. Tinwell, S. Colombel and R. Bars. *Research Toxicology, Bayer SAS, Sophia Antipolis CEDEX, France.*

In response to the demand for increased regulation of endocrine disrupters (EDs), assays that have been developed specifically for their identification (both in vitro and in vivo) are becoming part of the safety evaluation of chemicals. These assays have undergone extensive validation using known EDs and currently much focus is being placed on the activity of synthetic chemicals, particularly agrochemicals, in these tests. However, very little, if any, information is available concerning the activity of compounds in these ED assays that we knowingly and often willingly expose ourselves to on a daily basis (eg caffeine, vitamins, painkillers). To address this absence of data, various substances routinely taken, often on a daily basis, have been tested in vitro using the H295R steroidogenesis assay. This assay is part of the OECD level 2 battery of in vitro assays for endocrine disruption and is mandatory in the current US EPA Endocrine Disruptor Screening Program. The data generated in this in vitro assay is considered to provide an alert for potential in vivo endocrine effects via disturbance of sex steroid hormone synthesis and can ultimately contribute to the subsequent categorization of a compound as an ED. We tested each compound at concentrations which were not cytotoxic and which were relevant in terms of possible daily adult human exposure. Our data indicate that whilst some compounds, such as folic acid and sucrose gave no clear effects on progesterone, testosterone or estradiol synthesis following 48h incubation with the H295R cells, other compounds such as caffeine and vitamin C consistently induced clear, dose related increases in estradiol secretion under the same conditions of test. In contrast, vitamins B6 and B3 appeared to reduce estradiol secretion. Evaluation of additional compounds such as paracetamol, ibuprofen and aloe vera is on-going; however this first dataset indicates the importance of determining and understanding assay limitations before embarking on data interpretation.

PS 2352 BISPHENOL A DISRUPTS STEROIDOGENIC PATHWAYS AND ADRENAL AND SEX HORMONE SECRETION IN THE HUMAN ADRENOCORTICAL H295R CELL LINE.

A. Oskarsson[1], <\s>Ohlsson[1], C. Jansson[2] and E. Ullerås[1]. [1]*Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden and* [2]*Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Bisphenol A (BPA), a monomer used in production of plastics, has endocrine disrupting properties through a broad spectrum of mechanisms. It acts on the estrogen, androgen and thyroid hormone systems by interacting with hormone receptors. Furthermore, BPA has been shown to affect the synthesis of sex hormones while less attention has been paid to adrenocortical hormones. We have investigated the effects of BPA on steroid hormone synthesis in the human adrenocortical cell line H295R, which expresses all enzymes required for secretion of adrenocortical and sex hormones. Cells were treated with 0.01-100 μM BPA for 24 hours and levels of 12 secreted hormones and steroid intermediates were determined by ELISA and LC-MS/MS. A dose-dependent inhibition of 11-deoxycortisol, cortisol, DHEA, androstenedione and testosterone secretion was demonstrated with significant effects starting at 0.1 μM BPA. In contrast, the secretion of progesterone and estradiol was increased by BPA. Secretion of 11-deoxycorticosterone, corticosterone and aldosterone was reduced but only at the highest BPA concentration and 17α-OH-progesterone was not affected at all. The observed pattern of effects might be mediated by an inhibition of the steroidogenic enzymes CYP17A1 and CYP21A2 together with a stimulation of CYP19A1. Gene expression, analysed by quantitative RT-PCR revealed a significant down-regulation of *CYP17A1, CYP21A2* and *3βHSD2*, but only at the highest concentration of BPA. An up-regulation in expression of the *CYP19A1* gene was observed, although only at 1 and 10 μM BPA. We conclude that BPA disrupts adrenal steroidogenesis, with cortisol secretion as a sensitive target, as well as synthesis of sex hormones in the human H295R cell line. The effects can only partly be explained by effects on expression of steroidogenic genes.

PS 2353 METABOLITE PATTERNS TO DIFFERENTIATE DIRECT FROM INDIRECT HYPOTHYROID EFFECTS.

V. Strauss[1], E. Fabian[1], M. Herold[2], H. Kamp[1], M. Kapp[1], G. Krennrich[1], R. Looser[2], W. Mellert[1], A. Prokoudine[2], T. Walk[2], J. Wiemer[2] and B. van Ravenzwaay[1]. [1]*BASF SE, Ludwigshafen, Germany and* [2]*Metanomics, Berlin, Germany.* Sponsor: R. Parod.

BASF and metanomics launched a project to predict toxicological risks by measuring metabolite profiles in rat serum during a single repeated dose screening study. For that purpose, a comprehensive database (MetaMapÒ Tox) has been established. About 250 metabolites were measured in serum samples of Wistar rats after administration of about 450 pharmaceutical, chemical and agrochemical compounds for 7, 14 and 28 days. Sets of common metabolite level changes (metabolite patterns) were arranged to characterize several toxicological modes of action (MOAs). Following administration of several compounds known to induce hypothyroidism, metabolite patterns were established to differentiate between a direct effect (thyroperoxidase inhibition) and an indirect effect (increased thyroid hormone conjugation via induction of liver microsomal enzymes) on thyroid hormone homeostasis. These patterns were confirmed by contrast with those seen when L-thyroxine was administered to rats to induce hyperthroidism. When the thyroid is indirectly affected, hormone levels are not consistently changed which can make diagnosis of endocrine disruption more difficult. Consideration of additional metabolite patterns can be used to substantiate the suspicion of an indirect hypothyroid effect.

PS 2354 TCDD, PCB126, AND PCB153 AFFECT HYPOTHALAMIC GNRH AND KISS-1 SIGNALING *IN VITRO*, BUT NOT *IN VIVO*.

K. Solak, F. Wijnolts, M. van den Berg and M. van Duursen. *Toxicology, IRAS, Utrecht University, Utrecht, Netherlands.*

Dioxins and polychlorinated biphenyls (PCBs) are responsible for causing a variety of adverse effects on development and physiology of the reproductive system. However the molecular mechanisms of the action of dioxin and PCBs on hypothalamic Gonadotropin-releasing hormone (GnRH) and Kisspeptin (Kiss-1), two key regulators of reproduction, still remain unclear. In this study, we investigated the effects of TCDD, PCB126 and PCB153 on the hypothalamus using in vivo exposed adult female rats and a new in vitro immortalized adult hypothalamic rat cell line GnV-3. Unlike the commonly used murine GT1-7 cell line, GnV-3 cells express the aryl hydrocarbon receptor (AhR) similar to the vivo situation. A single oral dose of TCDD (100 μg/kg bw), PCB126 (1000 μg/kg bw) but not PCB 153 (500000 μg/kg bw), resulted in a significant increase in hypothalamic CYP1A1 mRNA level of 400 fold and 212 fold, respectively, in adult female rats after 72 hours. Hypothalamic GnRH and Kisspeptin mRNA expression was not significantly changed. In the GnV-3 cells, a 72-hour exposure to TCDD (10 nM), PCB126 (100 nM) but not PCB 153 (10 μM) caused a significant induction in CYP1A1 mRNA expression. In this model, TCDD, PCB126 and PCB153 exposure resulted in the induction of GnRH mRNA expression and secretion, while Kiss-1 mRNA expression remained unchanged. These in vitro data suggest that dioxins and PCBs may act on the GnRH expression in neuronal cells, but the role of the AhR in GnRH and Kiss-1 signalling needs to be studied further. Also, the effect of dioxins on GnRH signaling was not observed in vivo. The complex interactions of neurotransmitters and steroid hormones within hypothalamus in vivo might influence the effect of dioxin and PCBs on GnRH secretion. The in vivo relevance of the GnV-3 in vitro model is currently being studied using primary rat hypothalamic cultures.

PS 2355 TCDD INDUCES BIOMARKERS FOR ENDOMETRIOSIS IN HUMAN ENDOMETRIUM CARCINOMA CELL LINE.

K. van Ede, S. Stelloo, M. van den Berg and M. van Duursen. *Toxicology, IRAS, Utrecht University, Utrecht, Netherlands.*

Endometriosis is a gynaecologic disorder that affects up to 10% of the women in reproductive age. A suggested pathway leading to endometriosis involves overexpression of estrogen receptor (ER)-beta, subsequent suppression of ER-alpha and the progesterone receptor (PR) and upregulation of cyclooxygenase-2 (COX-2) and CYP19. Environmental contaminants like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have often been linked to increased susceptibility to endometriosis. This study investigated the interaction of the aryl hydrocarbon receptor (AhR) with the ER and the PR in the human endometrium carcinoma cell line (ECC-1). ECC-1 cells were exposed to DMSO, TCDD alone or co-exposed to TCDD and estradiol (E2) or progesterone (P4). Gene expression of ER-alpha, ER-beta, PR, COX-2, CYP19 and cytochrome P450 1A1 (CYP1A1) were studied. Single exposure of TCDD resulted in a significant induction of CYP1A1 and COX-2 and downregulation of PR mRNA levels. Gene expression of CYP19 could not be determined in unexposed ECC-1 cells but was upregulated after exposure to TCDD. These findings correlate with the suggested pathway leading to endometriosis. Opposite, TCDD exposure did not affect gene expression of ER-alpha and caused downregulation of ER-beta mRNA levels. Interestingly, co-exposure of TCDD with P4 or E2 showed similar effects as seen after single exposure, except for ER-alpha and ER-beta. Co-exposure with E2 did not affect ER-alpha and ER-beta expression whereas co-exposure with P4 resulted in upregulation of ER-beta mRNA levels and significant downregulation of ER-alpha. The changes in gene expression in the ECC-1 cell line upon co-exposure of TCDD with E2 or P4 were similar to changes observed in uteri of female Sprague-Dawley rats three days after a single oral dose of TCDD. Taken together, these data suggest that TCDD is able to cause changes in gene expression in endometrium cells that have been associated with endometriosis. However, the role of circulating hormones on TCDD induced effects needs to be studied further.

PS 2356 SODIUM PHENOBARBITAL: ASSESSMENT OF PUBERTAL DEVELOPMENT AND THYROID FUNCTION IN A COMBINED MALE AND FEMALE PUBERTAL ASSAY.

M. Blanck, H. Vincent, E. Debruyne, A. Veillon, E. Balme, C. Pallen and C. Langrand-Lerche. *Experimental Toxicology, Bayer SAS, Sophia Antipolis, France.* Sponsor: E. Debruyne.

Sodium Phenobarbital (PB) is a weak thyroid acting agent that enhances thyroid hormone clearance. We investigated the effects of PB on pubertal development and thyroid function in a combined male and female pubertal assay (Tier I test to detect potential endocrine-disrupting chemicals, US guidelines 890.1500 & 890.1450). From 30 time-mated female Sprague-Dawley rats which were received at gestation day 7 or 8, groups of 20 immature littermates per sex were administered 100 mg/kg/day PB or vehicle (corn oil) on PND 22-42 (females) or PND 23-53 (males). Parameters evaluated included body weights, clinical signs, vaginal opening (VO), preputial separation (PS), estrous cycle, hormones levels, gross pathology, organ weights and histopathology. In order to assess the potential effects of anesthesia on hormones levels, blood samples were collected from 10 animals/sex/group at the abdominal aorta under Isoflurane anesthesia or by decapitation as recommended in the guidelines. Body weights were unaffected by PB treatment. Increased salivation was noted in males and lethargy was observed in both sexes. The age at PS and VO was slightly delayed. A large number of control animals failed to achieve regular cycles during the monitoring period after VO. Therefore it was not possible to assess the effect of PB on estrous cyclicity. TSH levels were slightly higher and testosterone concentrations were lower than controls. T4 levels remained unchanged. Hemolysis was noted in blood samples collected by decapitation. However there were no major differences in hormones levels between sera collected either by decapitation or under Isoflurane anesthesia. The reproductive organ weights were not affected by treatment. Histopathology examination is on-going. In conclusion, this study showed evidence of weak endocrine effects of PB in the male and female pubertal assay. In addition, under the study design conditions, the young age of animals and duration of the study did not allow to assess estrous cyclicity.

PS 2357 ACTIVITY OF LARGE-MOUTH BASS ESTROGEN RECEPTORS IN RESPONSE TO MODEL COMPOUNDS.

R. E. Weil[1], D. S. Barber[2] and N. D. Denslow[2]. [1]*Pharmacology, University of Florida, Gainsville, FL and* [2]*Physiological Sciences, University of Florida, Gainesville, FL.*

Estrogenic compounds found in the aquatic environment have been shown to disrupt normal endocrine function in fish by interacting with their hormone receptors. Estrogen is involved in a wide array of physiological processes, including the synthesis of vitellogenin (vtg), the egg yolk precursor protein. In largemouth bass (LMB) estrogen signaling is mediated via three known estrogen receptors (ERα, ERβb and ERβa). Studies have shown that the ligand-activated transcriptional activity of ERs is dependent on the estrogen response element (ERE) sequence in the promoters responsive genes. The first aim of this study was to use transfection assays to determine 1) if increasing the number of tandem EREs (from two to three) in the promoter of a reporter gene increases transcriptional response of each of the ERs to estrogen, and 2) to test the ability of the three LMB ERs to respond to 4,4',4"-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol (PPT) a mammalian ERα specific agonist, 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN), a mammalian ERβ specific agonist, bisphenol A (BPA), pp'-DDE, dieldrin (model environmental estrogens), and ICI182,780. The second aim is to determine the effects of these compounds on vtg synthesis using an *in vitro* assay. The results indicate that three tandem EREs in the promoter region of the reporter gene leads to a higher E2-me-

diated transcriptional response of all three ERs as compared to the two tandem EREs. PPT is not a specific agonist for the LMB ERα, as it also activates ERβ, and DPN was shown to activate the ERβs as well as the ERα. As expected, pp'-DDE and BPA were weak agonists of all three receptors. Dieldrin appears to be a more specific agonist of ERα, as it is not very efficacious in activating either ERβb or ERβa. The three LMB ERs showed different sensitivities to ICI 182,780, but at high concentrations (>5μM) it was able to completely inhibit the E2-mediated activation of all three ERs. Tissue slices from LMB livers will be used to test the effects of the above mentioned compounds on vtg synthesis.

PS 2358 IMPACT OF OSRI ON ANIMAL USE AND SCREENING BURDEN FOR TIER I EDSP COMPLIANCE.

S. C. Gehen[1] and W. R. Jones[2]. [1]*Dow AgroSciences, LLC, Indianapolis, IN and* [2]*CropLife America, Washington, DC.*

In response to provisions of the Food Quality Protection Act (FQPA), EPA developed the endocrine disruptor screening program (EDSP) with the intent to identify chemicals that might interact with estrogen, androgen, or thyroid systems. In late 2009, EDSP was implemented with receipt of testing orders for the chemicals on the first screening list, the majority being pesticide active ingredients. According to FQPA language and EPA policy, registrants can utilize existing toxicology information- or other scientifically relevant information (OSRI) - in lieu of generating new data for some or all tier I assays. This policy is especially noteworthy for pesticides where existing legislation mandates a comprehensive registration package of toxicology studies, several which assess endocrine-sensitive parameters. To evaluate the impact of OSRI on animal use and overall burden of endocrine screening, a retrospective analysis of registrant and EPA responses was conducted. Of the 58 individual pesticide registrants and consortia receiving testing orders, about 10% opted to voluntary cancel their respective EPA registration. Of those remaining, the degree to which registrants utilized OSRI was variable with 34% submitting OSRI for all 11 tier I assays, 47% for a sub-set of tier I assays, and 9% choosing to conduct all 11 assays. Registrants cited or submitted OSRI for a total of 211 (61%) in vivo EDSP studies; these responses ranged from 76% citing OSRI for the male pubertal assay to just 47% for the fish short-term reproduction assay. Of the first 5 test order responses published by EPA, registrants cited OSRI for 14 out of a possible 30 in vivo assays; however, none of these data-sets were considered sufficient by EPA to satisfy the test order, thus indicating that new in vivo studies would be required. Understanding the agency's rationale for accepting or rejecting data submitted as OSRI will be increasingly important as this rationale directly affects animal use and the overall resource burden of the EDSP program.

PS 2359 ORAL EXPOSURE TO BISPHENOL A IMPACTS ELECTROCARDIOGRAPHIC PARAMETERS IN CD-1 MICE.

C. B. Lo, T. B. McCutchan, E. L. Kendig and S. M. Belcher. *Pharmacology, University of Cincinnati, Cincinnati, OH.*

Bisphenol A (BPA) is an estrogenic endocrine disrupting chemical that is prevalent in the environment. Humans are exposed to significant amounts of BPA from the consumption of contaminated foods, beverages and other poorly defined routes. Our research has demonstrated that BPA can affect the cardiovascular system by altering intracellular Ca2+ handling in myocytes causing altered EC coupling and arrhythmias. The aim of this study was to determine whether oral exposure to estradiol or BPA can influence normal electrical function and rhythm of the heart in live CD1 mice. Male and female CD-1 mice were randomly paired following a two week pre-treatment period during which time animals were exposed to control diet or diet containing BPA (0.03, 0.3, 3, 30, and 300 ppm) or 17α-ethinyl estradiol (EE; 0.0001, 0.001, and 0.01 ppm). Treatments were continued through the life of breeders (F0) and offspring (F1). Electrocardiogram (ECG) data was collected and analyzed from conscious and unrestrained mice at different time points prior to mating, during pregnancy, and during offspring development. Because levels of endogenous estrogen vary during the estrous cycle, the stage of estrous at time of recording was determined. No significant differences in heart rate or other ECG parameters were observed during estrous in the control group. Dietary exposure to BPA (0.03 ppm and 0.3 ppm) and EE (0.01 ppm) caused significant effects on cardiovascular function during diestrus and estrus in CD-1 mice. The most efficacious dose of BPA was 0.3 ppm, while no effects have been observed at 30.0 ppm BPA. In F0 females 0.03 ppm BPA significantly increased heart rate and heart rate variability was suppressed in males. In the offspring (F0), BPA (0.03 ppm and 0.3 ppm) and EE caused significant effects on cardiovascular function during diestrus and estrus. For these endpoints the most efficacious dose of BPA was 0.3 ppm, with no effects observed at 30.0 ppm BPA.

PS 2360 INTEGRATING HUMAN AND TOXICOLOGICAL EVIDENCE TO UNDERSTAND PCB EFFECTS ON THE DEVELOPING BRAIN.

F. Parham[1], A. Wise[2], D. A. Axelrad[3], K. Z. Guyton[3], C. J. Portier[4], L. Zeise[5], R. T. Zoeller[6] and T. J. Woodruff[2]. [1]*National Institute for Environmental Health Science, Research Triangle Park, NC,* [2]*University of California, San Francisco, CA,* [3]*U.S. EPA, Washington, DC,* [4]*Centers for Disease Control and Prevention, Atlanta, GA,* [5]*California EPA, Oakland, CA and* [6]*University of Massachusetts, Amherst, MA.*

We reviewed the relationships between chemical exposure, early biological disturbances, and subsequent overt health effects for thyroid hormone disruptions in a case study approach. Thyroid hormones (TH; thyroxine, T4 and triiodothyrine, T3) are essential for normal brain development and for normal physiological controls in adults. Mechanistic, laboratory animal, and human studies indicate that TH disruption during the prenatal period can cause permanent cognitive and neurobehavioral deficits. The quantitative relationships between TH disruption due to chemical exposures and overt disease were analyzed for polychlorinated biphenyls (PCBs). PCBs were chosen because sufficient animal and human data exist to model and compare body burdens, TH disruption and health decrements. We modeled three relationships following a review of 13 epidemiologic studies, applying meta-analysis where appropriate: 1) chemical exposures and TH changes; 2) pregnancy TH changes and neurodevelopmental outcomes of children; and 3) prenatal PCB exposures and neurodevelopmental outcomes of children. Using data from four chronic PCB studies in rats conducted by the National Toxicology Program, we developed a low-dose linear model of PCB body burdens and T4 (free and total) decrements, accounting for 35 PCB congeners. A human extrapolation of the animal findings generally under-predicted the dose-response estimated from human studies. This quantitative analysis can inform approaches for evaluating human health consequences of exposures to multiple chemicals that exert thyroid toxicity.
Disclaimer: The views expressed are those of the authors and do not necessarily represent the views and/or policies of the US EPA.

PS 2361 GENISTEIN NEGATES THE INHIBITORY EFFECTS OF LETROZOLE ON AROMATASE RELATED TO MALIGNANT, BUT NOT HEALTHY BREAST TISSUE.

M. van Duursen[1], E. de Morree[1], S. Nijmeijer[1], P. de Jong[2] and M. van den Berg[1]. [1]*Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands and* [2]*Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, Netherlands.*

Aromatase (cytochrome P450 19, CYP19) catalyzes the conversion of androgens into estrogens. Especially in post-menopausal women, estrogen production in breast tissue by CYP19 becomes a major site of hormone production. In healthy breast adipose fibroblasts (BAF), CYP19 expression is low and is mainly driven by promoter I.4. In fibroblasts surrounding a tumor, a promoter switch to PI.3/II takes place and CYP19 expression is elevated 3-4-fold. This makes this enzyme an important target for estrogen-dependent breast cancer treatment by aromatase inhibitors, such as letrozole (LET). Breast cancer treatment often results in sudden onset of menopausal symptoms and many women seek for alternative therapies to treat these symptoms. A commonly used treatment is the soy-isoflavone genistein (GEN). In this study, the potential interaction between LET and GEN on CYP19 was studied. In healthy primary human BAFs, PI.4-driven CYP19 expression was increased 45-fold by its stimulant dexamethasone. Expression of PI.3/II-driven aromatase was low in BAFs and only increased 7-fold by the stimulant PGE2. GEN did not affect CYP19 expression in BAFs. In contrast, in human adrenocorticocarcinoma H295R cells, that express PI.3/II-driven CYP19, GEN concentration-dependently induced aromatase activity (EC50=26 μM) and expression. LET inhibited CYP19 activity in H295R cells with an IC50 value of 0.2 μM. Co-incubation with GEN resulted in a concentration-dependent negation of CYP19 inhibition by LET through increased CYP19 expression. This interaction did not affect the EC50 of genistein, nor did it alter the IC50 of LET. These data indicate that the actions of GEN on CYP19 appear to be restricted to PI.3/II-driven CYP19 that can be found in BAFs surrounding a tumor. Therefore, the use of GEN by women undergoing breast cancer treatment with LET should be considered with care.

PS 2362 EFFECTS OF REDUCED-PHYTOESTROGEN DIETS ON DEVELOPMENT OF CD1 AND C57BL/6N MICE.

E. L. Kendig, S. M. Christie, D. R. Buesing, C. K. Cookman, R. B. Gear, C. B. Lo and S. M. Belcher. *Pharmacology, University of Cincinnati, Cincinnati, OH.*

Endocrine disrupting chemicals (EDC) are chemicals that can affect endocrine-dependent development and function. These chemicals are prevalent in the environment and exhibit a variety of biological effects. Because of limited ability to replicate results from some animal studies controversy surrounds the possible harmful

effects of bisphenol A. Two factors contributing to such differing results include highly variable estrogen-like contamination and the exquisite sensitivity of endocrine systems. In rodent studies possible environmental factors affecting background EDC levels includ: types of caging, bedding, and sanitation materials, drinking water and diet. Laboratory rodent diet remains a significant uncontrolled variable in many experiments assessing the effects of EDCs. Previously, Thigpen et al. demonstrated that dietary formulation and mill dates of rodent chow impact sensitivity of end-points of estrogen-like activity. The purpose of the current study was to determine the optimal "reduced EDC" diet for consistent detection of the effects of estrogen-like EDC exposure. Treatment-related differences in reproductive and developmental endpoints were observed among groups fed four diets having reduced phytoestrogen content. Animals were continuously fed from 2 weeks before copulation (F0 treatment) until sacrifice with offspring (F1) treated throughout life. A positive control diet with 17α-ethinylestradiol was included. Changes were observed in onset of puberty, organ weights, and adipose deposition. These findings support the proposal by Thigpen et al. that caloric content of the diet is a confounder in EDC exposure studies and reveal that a reduction of diet caloric content and elimination of EDC phytochemicals can reduce diet-induced study variability to yield increased reproducibility of animal studies investigating actions of EDCs.

PS 2363 DEVELOPMENTAL TOXICITY OF O-METHYLATED METABOLITES OF BISPHENOL A.

K. C. Pangallo, E. Kirichenko, K. Cooper and L. A. White. *Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ.*

Bisphenol A (BPA), an estrogen agonist and known endocrine disruptor, is a common component of plastics and epoxy resins. Migration of BPA out of commercial products results in environmental contamination, and provides opportunity for human exposure to BPA and biotransformation to BPA metabolites. Recent work in our lab has demonstrated O-methylation of BPA to monomethyl and dimethyl ether metabolites (BPA-MME and BPA-DME, respectively) under aerobic conditions by soil-dwelling Mycobacterium species. This transformation results in increased toxicity, as BPA-MME and BPA-DME have lower LC50s than their parent compound to developing zebrafish (Danio rerio) embryos. Here we investigate the mechanism of this increased toxicity, and show that the O-methylated metabolites are stronger inducers of matrix metalloproteinases (MMP-2, -9 and -13) than the parent compound during D. rerio development. The increased toxicity of O-methylated metabolites, and the ubiquity of organisms capable of O-methylation, suggests that this transformation pathway must be considered when making a risk assessment of BPA exposure.

PS 2364 SERINE PROTEASE INHIBITOR, AEBSF, STRONGLY IMPAIRS EXPRESSION OF PROINFLAMMATORY IFNγ AND RELATED BIOMOLECULES FROM SPLENOCYTES FROM ESTROGEN-TREATED C57BL/6 MICE.

E. Karpuzoglu[1], R. M. Gogal[1] and A. Ahmed[2]. [1]*Department of Anatomy and Radiology, University of Georgia, Athens, GA and* [2]*Department of Biomedical Sciences and Pathobiology, Virginia Maryland Regional College of Veterinary Medicine, Blacksburg, VA.*

Estrogen is shown to alter immune responses and associated with enhanced susceptibility to autoimmunity in females. We have demonstrated that estrogen enhances proinflammatory IL-12, IFNγ, iNOS, and MCP1, that has a critical role in Th1 profile as well as female predominant autoimmune diseases. However, the immune modulation of estrogen mediated inflammatory responses remains to be elucidated. In this study, the immunomodulatory effects of a serine protease inhibitor, 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), on estrogen-mediated induction of inflammatory cytokines (IFNγ, IL-6, MCP1, iNOS, NO) and related transcription factors (STAT1) in ConA or rIL-12-treated splenocytes are explored. AEBSF was found to downregulate the estrogen-induced proinflammatory IFNγ, IL-17, IL-6, MCP1, iNOS expressions from ConA-activated splenocytes. The AEBSF-derived reduction of IFNγ and MCP1 were also observed upon IL-12receptor specific stimulation with Th1-inducing rIL-12 after 24 h from splenocytes from estrogen-treated mice. The cell cycle analysis showed no marked change in phases of cell cycle (G1/G0, S, or G2/M) due to rIL-12 and/or AEBSF treatment in splenocytes from estrogen and placebo-treated mice after 24h of culture. Furthermore, AEBSF alters the estrogen-enhanced expression IFNγ and STAT1 expression but did not markedly affect IRF1 protein levels at 3h compared to placebo controls. These novel findings indicate that AESBF could alter estrogen-mediated proinflammatory events in the immune system, and thus might lead to new avenues in regulation of autoimmune diseases.

PS 2365 LOW-DOSE BPA ALTERS CA CYCLING AND PROMOTES ARRHYTHMOGENESIS IN THE FEMALE HEART.

S. M. Belcher, S. Yan, Y. Chen, M. Dong and H. Wang. *Pharmacology, University of Cincinnati, Cincinnati, OH.*

There is wide-spread human exposure to the ubiquitous estrogenic chemical bisphenol A (BPA) and growing evidence implicating BPA as a significant concern to human health. Epidemiologic studies have demonstrated that higher urine BPA concentrations are associated with cardiovascular diseases in humans. However, the nature and underlying mechanism of the cardiac effects of BPA exposure are unknown. In this study, we examined the rapid effects of BPA and 17α-estrodial (E2) on rodent ventricular myocytes and whole hearts and the underlying mechanisms. Physiological low-dose (10-9 M) of BPA or E2 rapidly induced arrhythmogenic triggered activities in isolated myocytes from female, but not male, rat hearts. The effects of BPA were particularly pronounced with combined with E2. BPA and E2 also promoted ventricular arrhythmias, particularly malignant ventricular fibrillation under catecholamine-induced stress in female, but not male, rat whole hearts. The dose-response curves for both BPA and E2 have an inverted-U shape, with the most efficacious doses at 10-9 M. The cellular mechanism of the pro-arrhythmic effects of BPA and E2 were investigated. The estrogens rapidly enhanced sarcoplasmic reticulum (SR) Ca2+ load and release, and markedly increased frequency of Ca2+ sparks, or spontaneous SR Ca2+ "leak". Pharmacological blockade of SR Ca2+ leak suppressed estrogen-induced triggered activities. Ablation of ERβ in an ERβ knockout mouse model completely abolished triggered activities induced by estrogens in female myocytes. In conclusion, low-dose of BPA and E2 rapidly promote arrhythmias in the heart in a female-specific manner; the pro-arrhythmic actions of estrogens are mediated by ERβ-signaling through alterations of myocyte Ca2+ cycling, particularly increases in Ca2+ leak from the SR.

PS 2366 ARSENITE INHIBITS 3T3-L1 ADIPOGENESIS AND SUPPRESSES INDUCTION OF NUCLEAR RECEPTORS DURING THE EARLIEST STAGES OF DIFFERENTIATION.

F. J. Zandbergen[1], V. Chatikavanij[1], C. D. Kozul-Horvath[2] and J. W. Hamilton[1]. [1]*Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA and* [2]*Department of Immunology, Dartmouth Medical School, Lebanon, NH.*

Exposure to arsenic (As) is associated with an increased risk of serious illnesses, including cardiovascular disease (CVD). Obesity, defined as excess adipose tissue, is associated with elevated plasma triglyceride levels, a strong risk factor for CVD. We have previously shown that arsenite (AsIII) can suppress the activation of steroid receptors, which are part of the nuclear receptor family. We hypothesized that AsIII might affect lipid metabolism through targeting other members of this family as well. Since adipose tissue plays a major role in lipid homeostasis, we used the mouse 3T3-L1 cell line, which can be induced to differentiate into adipocytes, for our studies. AsIII markedly suppressed mRNA expression of several markers of adipogenesis in a concentration dependent manner. In addition, expression of nuclear receptors that are induced very early in 3T3-L1 differentiation was significantly suppressed. Correspondingly, AsIII treatment from the time of differentation induction resulted in a concentration dependent decrease in lipid accumulation. But when AsIII treatment was started several days after the cells had been induced to differentiate, expression of adipogenic markers was increased. These results show that depending on cell differentiation stage AsIII may have different, or even opposite effects on adipogenesis, and that inhibition of adipogenesis may be related to suppression of nuclear receptors very early in 3T3-L1 differentiation. We speculate that *in vivo*, the effects of AsIII on adipogenesis might decrease lipid storage capacity, which could be disadvantageous under obesity promoting conditions if the result is an elevated level of plasma lipids. Interference of AsIII with lipid metabolism in differentiated adipocytes might also change plasma lipid levels, likewise providing a possible explanation for the association of AsIII with increased risk for CVD. (NIH-NIEHS P42 ES007373)

PS 2367 SCREENING, CLASSIFICATION, AND MECHANISM OF ACTION OF ENDOCRINE DISRUPTING CHEMICALS.

X. Wang, G. Du, O. Shen, H. Sun, L. Xu, H. Chen, Y. Xia, A. Gu and S. Wang. *Nanjing Medical University, Institute of Toxicology, Nanjing Jiangsu, China.*

The potential effects of substances with endocrine activities in humans and wildlife, so-called endocrine-disrupting chemicals (EDCs), constitute a major concern among the public and the scientific community. Screening of chemicals for endocrine disrupting (ED) activity is a major goal of research groups and regulatory agencies worldwide. Our laboratory have set up a battery of assays to detect the en-

docrine disruptor activities of EDCs including ER / AR/ TR mediated reporter gene assay, E-screening / A-screening / T-screening, pS2 mRNA expression assay, ER competitive-binding assay, amphibian metamorphosis (Xenopus laevis), steroidogenesis (H295R cell line). Combining these assays, we can detect chemicals with (anti)estrogenic, (anti)androgenic and (anti)thyroid hormone activities effects, and identify chemicals that affect steroidogenesis. We have screened several chemicals including industrial chemicals and pesticides, such as alkylphenols, bisphenol A and its halogenated derivatives, phthalates, pyrethroids, perfluorinated chemicals, carbaryl and related metabolites. Use this screening battery, we can primarily screen and classify EDCs into estrogenic, androgenic and thyroid hormone and steroidogenesis disruptors. These sensitive assays are capable of high-throughput processing and provide mechanistic insight. We also explored the potential molecular mechanisms of TR disruption. We found that phthalates could affect the metamorphic development of X. laevis by affecting the gene expression, enhanceing the interactions between co-repressor Silence Mediator of Retinoic acid and Thyroid hormone (SMRT) and TR, changing the methylation status of promoter region of TRβ gene. Take together, our studies could provide some useful information for the assessment of environment chemicals on human health.

PS 2368 EFFECTS OF CHOLESTEROL-ALTERING PHARMACEUTICALS ON CHOLESTEROL METABOLISM, STEROIDOGENESIS, AND GENE EXPRESSION IN THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*).

S. Y. Skolness[1, 2], J. Cavallin[2], E. Durhan[2], K. Jensen[2], M. Kahl[2], C. LaLone[2], E. Makynen[2], D. Villeneuve[2], L. Wehmas[2] and G. Ankley[2]. *[1]University of Minnesota, Duluth, MN and [2]U.S. EPA Mid-Continent Ecology Division, Duluth, MN.*

Pharmaceuticals that target cholesterol biosynthesis and uptake are among the most widely prescribed drugs and have been detected in the aquatic environment. Fibrates are a class of pharmaceuticals that indirectly modulate cholesterol biosynthesis through effects on peroxisome proliferator-activated receptors. Statins lower endogenous cholesterol production by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate limiting step in cholesterol synthesis. The objective of the present study was to assess the physiological and reproductive impacts of a fibrate, gemfibrozil, and a statin, rosuvastatin, on adult fathead minnows (*Pimephales promelas*). Fathead minnows were exposed to gemfibrozil or rosuvastatin in three different studies. Cholesterol, triglyceride, vitellogenin, and sex steroid (testosterone (T), 17β-estradiol (E2)) concentrations were determined in the plasma. Expression of a number of cholesterol metabolism-related genes in liver and steroidogenesis-related genes in the gonad was determined. Ex vivo production of T and E2 by gonad tissue was also determined. Gemfibrozil significantly lowered plasma cholesterol concentrations in the males exposed to 600 μg/L for 8 days. Expression of several genes important to lipid metabolism was significantly altered, suggesting that gemfibrozil does affect lipid metabolism in fish. There was also a significant reduction in male ex vivo T production after 2 days of exposure to gemfibrozil. In addition, a 21 day reproduction study with gemfibrozil was completed to further investigate the effects observed in the male fathead minnows and their potential implications for fish reproduction. This study adds to our knowledge of possible effects of common pharmaceutical pollutants on fish.

PS 2369 RETINOIC ACID MODULATES AHR-MEDIATED EFFECTS OF TCDD IN OSTEOBLASTIC CELLS.

M. Herlin[1], R. Heimeier[1], M. Korkalainen[2], M. Viluksela[2] and H. Håkansson[1]. *[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and [2]National Institute for Health and Welfare, Kuopio, Finland.*

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that can be activated by a range of structurally diverse chemicals, including ubiquitous environmental contaminants such as dioxins. An increasing number of studies show that exposure to high affinity AhR ligands, such as TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin), can induce alterations in bone geometry, bone mineral density and bone mechanical strength. Exposure to such exogenous hormonally active substances can thus have deleterious implications to human health. This study proposed to identify critical AhR-modulated signaling pathways that may be disrupted in bone by TCDD based on molecular analysis in vitro. Retinoic acid (RA) signaling is one such pathway of interest as retinoid metabolism seems to be involved in TCDD-induced toxicity. RA is a lipid soluble hormone, which exerts a wide variety of effects on development, cellular differentiation and homeostasis in various tissues, including bone growth and development. Currently the role of RA in bone toxicity is unexplored. In this study, we exposed MCT3T-E1 cells, an osteoblast precursor cell line, to TCDD in the presence or absence of all-trans RA or 9-cis RA. Both RA isoforms showed a protective effect against TCDD-induced AhR activation. These results suggest that RA could modulate AhR-mediated effects of TCDD in differentiating osteoblastic cells. Our finding that RA inhibits AhR-pathways that affect bone differentiation in vitro thus provides a novel mechanism for the toxicity effects of dioxins on bone development. Further studies are needed to delineate the molecular mechanisms involved and potential crosstalk between AhR and RA signaling pathways.

PS 2370 DETERMINING STRUCTURAL DETERMINANTS OF PHTHALATE ANTIANDROGENIC POTENCY *IN VITRO* USING RAT AND MOUSE LEYDIG TUMOR CELLS.

R. A. Clewell, K. Edwards, J. Campbell, H. Clewell and M. Andersen. *The Hamner Institutes, Research Triangle Park, NC.*

Some phthalate esters (PEs) disrupt sexual development in the male rat after gestational exposure, which is at least partially due to reduced testosterone (T) synthesis in the fetal Leydig cell. We previously developed a mouse Leydig cell (MA-10) assay to test antiandrogenic activity of the PEs *in vitro*. However, several important differences exist between this cell line and cells affected *in vivo*. MA-10s are derived from the adult mouse and require luteinizing hormone (LH) receptor stimulation for testosterone (T) production. *In vivo* antiandrogenic effects are only seen in the rat, after fetal exposure, and fetal T is not regulated by LH. Thus, we explored whether a rat tumor derived cell line (R2C) that produces T independent of LH would provide an accurate and more relevant model for PE inhibition. A variety of PEs were tested in both cells, with alkyl chains from 1 to 9 carbons (C1 - C9) in length and various degrees of branching. RT-PCR was performed for 4 genes typically down-regulated in the fetal testis after PE exposure (*Cyp11a1*, *Cyp17a1*, *StAR* and *Scarb1*). All four genes were down-regulated in MA-10s after MEHP treatment, while only *Cyp11a1* and *Cyp17a1* were reduced in the R2Cs. While R2Cs were somewhat less sensitive to T inhibition, the trend across PEs was the same in the two cell lines, indicating that these *in vitro* assays are useful for predicting PE relative activity *in vivo*. In agreement with *in vivo* studies, T synthesis in both cell lines was inhibited by monoethylhexyl (MEHP) and monobutyl (MBP), but not monoethyl phthalate (MEP). EC_{50}s were 4 and 16 uM (MEHP), 3 and 12 uM (MBP) and ≥ 100 uM (MEP) in the MA-10 and R2Cs, respectively. In both cell lines, potency was highest in PEs with alkyl chain backbone lengths of 4-5 carbons. In longer chain PEs, branching tended to increase potency for T inhibition.

PS 2371 ISOLATION AND CHARACTERIZATION OF CHEMOPREVENTATIVE ANALYTES FROM AN ACETONE EXTRACT OF *KOLA ACUMINATA.*

J. Wynder[1, 2] and W. G. Gray[1, 2]. *[1]Chemistry, Southern University A&M College, Baton Rouge, LA and [2]Environmental Toxicology, Southern University Baton Rouge, Baton Rouge, LA.*

The search for effective treatment and potential eradication of prostate and breast cancer has seen a merger between the use of synthetic chemo-preventative agents and natural dietary products. The World Health Organization has recognized that more than fifty percent of scientifically developed drugs are either natural products, a derivative of natural products, or inspired by natural products. Our laboratory has been engaged in characterizing the androgenic and "putative" chemo-preventative properties of the common Jamaican bush tea "bizzy" nut (*Kola acuminata*, also known as obi) using the androgen responsive (LNCaP-AR+) and estrogen responsive (MCF-7-) cell lines.. The goal of this research project is to test the hypothesis that Biz-E3 contains a unique set of analytes that modulates cancer cell function. The specific objective, of this study was to generate Biz-E3 and Biz-E5 specific chemoinformatic libraries with its associated gene expression profile data. To accomplish this objective a High Performance Liquid Chromatograph (HPLC) and UV-Vis spectroscopy profiles for Biz-E3 and Biz-E5 were generated. We examined the expression of key regulatory genes along the apoptotic pathway in the presence of these extracts. Characterization of Biz-E3 by HPLC revealed the presence of seven distinct peaks, all eluting within the first 32 minutes of the HPLC run. Biz-E5 showed fewer peaks as compared to Biz-E3. The wavelength spectra of selected Biz-E3 HPLC peaks detected the presence of 4 to 5 individual analytes per peak. The crude Biz-E3 extract and selected HPLC fractionations were tested in growth-inhibitory assays in the MCF-7 cells and found to have strong anti-estrogen properties. Our results suggest that Bizzy nut contains unique analytes may be responsible for the "putative" chemo- preventative effects reported for this natural product.

PS 2372 EFFECT OF NOREPINEPHRINE, ENDOGENOUS DOPAMINE, AND EXOGENOUS DOPAMINE ON PROLACTIN RELEASE IN AN OVARIECTOMIZED RAT MODEL.

D. A. Brott[1], P. Campbell[1], P. Bentley[1], H. Andersson[2], J. Stewart[3], R. Huby[3] and L. Kinter[1]. [1]*Global Safety Assessment, R&D, AstraZeneca Pharmaceuticals, Wilmington, DE,* [2]*Global Safety Assessment, R&D, AstraZeneca Pharmaceuticals, Södertälje, Sweden and* [3]*Global Safety Assessment, R&D, AstraZeneca Pharmaceuticals, Alderley Park, United Kingdom.*

Dopaminergics (D) can produce unwanted endocrine side-effects in rats; D2 receptor agonists inhibit prolactin release. To characterize the mechanism, this study evaluated effects of central-acting: 1) exogenous dopamine (dopamine agonist, bromocriptine), 2) endogenous dopamine (dopamine transporter, DAT inhibitors, mazindol and GBR12909) and 3) endogenous norepinephrine (NE, NE transport inhibitor, nisoxetine), and 4) peripheral-acting exogenous dopamine (dopamine agonist, carmoxirole) on prolactin release using double cannulated ovariectomized rats. Rats were dosed with estradiol (E2, 0.6 - 20 ug/rat, iv), blood collected every 30 minutes between 1.5 to 5 hours, and evaluated for prolactin and luteinizing hormone (LH). E2 at 2 ug/rat (iv) was deemed the optimal concentration based on maximum prolactin release and expected suppression of LH. In remaining experiments, rats were dosed with E2 (2 ug/rat, iv) one hour prior to compound treatment (iv), blood collected 0.5 to 4 hours, and evaluated for prolactin. As expected the D2 receptor agonist bromocriptine (1 mg/kg) inhibited the E2-induced prolactin release, and so did the DAT inhibitors mazindol (5 mg/kg) and GBR12909 (3 mg/kg). Carmoxirole (15 mg/kg) induced a rapid initial release of prolactin, and inhibited E2-induced release. E2-induced prolactin release was not inhibited by the central acting NE uptake inhibitor nisoxetine (10 mg/kg). This study qualified the double cannulated ovariectomized rat model for evaluating the impact of central and peripheral acting compounds, and suggests that the mechanism may be activated both inside and outside the blood-brain barrier with D but not NE.

PS 2373 IDENTIFICATION OF ENDOCRINE DISRUPTORS USING AN ORGANOTYPIC VAGINAL TISSUE MODEL.

S. Ayehunie, K. LaRosa, T. Landry, J. E. Sheasgreen and M. Klausner. *MatTek Corp, Ashland, MA.* Sponsor: P. Hayden.

Environmental or occupational exposure to a broad variety of chemical agents can alter normal endocrine function. The effects of these "endocrine disruptors" (ED) can have serious health implications including deleterious effects to reproductive capacity, fetal development, the immune system, and carcinogenesis. Current animal tests are expensive, use a large number of animals, and are not necessarily applicable to humans. In addition, the Cosmetics Directive bans the use of animals for studies involving a broad variety of cosmetic and personal care products. In this study, we investigated the potential use of an organotypic tissue model, EpiVaginal™, for Tier 1 screening of chemicals that may be agonists or antagonists of the estrogen receptor (ER). MTT, H&E staining, RT-PCR, and ELISA assays were used to define tissue viability, structure, gene expression, and estrone release patterns, respectively. Based on the MTT studies, only concentrations resulting in >50% tissue viability were used. H&E stained tissue cross-sections showed thinning of the basal and parabasal layers following 72 hour exposure to ER antagonists when compared to the control tissues; exposure to ER agonists resulted in thicker basal and parabasal layers, indicating stimulation of cellular proliferation. RT PCR analysis showed an increase in progesterone receptor B (PRb) levels for 3 of 3 agonists and a decrease or no change for 6 of 8 antagonists when compared to negative controls. Furthermore, Er-α expression increased following exposure to the ER agonists and decreased following exposure to ER antagonists. ELISA assays showed increased estrone release by ED agonists but not ED antagonists. Based on estrone release (n=22 test articles), a prediction model (PM) for ER agonists was established. The PM identifies ER agonists with a high sensitivity (85.7%), specificity (100%), and accuracy (95.5%). In conclusion, the EpiVaginal tissue appears to be a useful in vitro model to screen for chemicals with endocrine disrupting potential.

PS 2374 EVALUATION OF SUBCHRONIC TOXICITY AND ESTROGENIC ACTIVITY OF BLACK COHOSH IN FEMALE WEANLING B6C4F1 MICE EXPOSED BY GAVAGE.

M. Mercado-Feliciano[1], M. D. Stout[1], C. A. Granville[2], M. Hejtmancik[2], R. Newbold[1], M. K. Vallant[1] and P. M. Foster[1]. [1]*National Toxicology Program, NIEHS, Research Triangle Park, NC and* [2]*Battelle Memorial Institute, Columbus, OH.*

Black cohosh (Actaea racemosa) is a popular herbal supplement for the treatment of gynecological symptoms. The recommended dose is 40 mg/day (~0.5–0.6 mg/kg/day). The current scientific literature suggests black cohosh extract may reduce luteinizing hormone secretion in ovariectomized rats, but demonstrations of estrogenic activity remain inconclusive. The National Toxicology Program (NTP) is currently assessing the possible toxicity and estrogenic effects of black cohosh in in vivo rodent models. We previously reported that a 90-day NTP study showed a 3-day delay in vaginal patency in Wistar Han rats, suggesting the possibility of anti-estrogenic activity. In addition, the thymus was the only major target organ in the rat study. However, no significant estrogenic effects were observed in an immature CD-1 mouse uterotrophic assay at up to 100 mg/kg/day delivered by subcutaneous injection (higher doses caused acute toxicity). A more recent NTP 90-day study was conducted in weanling female B6C3F1 mice administered an ethanolic extract by gavage (in 0.5% aqueous methyl cellulose) of 0, 62.5, 125, 250, 500 or 1000 mg/kg/d black cohosh. As in the previous rat study, time to vaginal patency and analysis of estrous cyclicity were included in addition to the routine toxicity endpoints. There was no apparent in-life toxicity following exposure. A dose related increase in platelets was statistically significant only for the 250 and 1000 mg/kg groups, similar to that seen in the rat. Increased liver weights were observed at 500 mg/kg and 1000 mg/kg, a trend very similar to that observed previously in rats. A 2-day delay in vaginal patency at 125, 250 and 1000 mg/kg was not statistically significant. With the exception of increased liver weight, we observed no other systemic effects and no evidence of estrogenic activity in mice.

PS 2375 IMPLEMENTATION OF TIER I MAMMALIAN ASSAYS FOR THE U.S. EPA ENDOCRINE DISRUPTOR SCREENING PROGRAM (EDSP).

S. Papineni[1], A. Tobia[2] and M. S. Marty[3]. [1]*Dow AgroSciences, Indianapolis, IN,* [2]*Nufarm Americas, Inc., Cary, NC and* [3]*The Dow Chemical Company, Midland, MI.*

Tier I screening under the EPA EDSP has begun for compounds on the first priority list. For the mammalian Tier I EDSP screens, dose level setting is a critical variable that can affect assay outcome. However, dose selection is challenging as each assay uses animals of different ages that are intact, peripubescent or castrated. Furthermore, there are little or no previous data on gavage dosing in animals at these young ages and the definition of maximum tolerated dose (MTD) varies by assay. One approach is to conduct a two-week probe study via gavage in which male and female rats (4-5/dose) are exposed to test compound from PND 22-36 (females) or 30-44 (males), then clinical observations, body weight, clinical pathology parameters and organ weights are examined. Histopathology may be included depending on the compound and its target organ. These data, coupled with previous toxicity data from the OSRI document (if available), can be used to select dose levels for Tier I assays, potentially using fewer animals than other approaches. Once doses are selected, use of MTD doses may raise issues with assay specificity. For example, the Hershberger assay is designed to detect compounds that interact with the androgen receptor (AR) or inhibit 5-alpha-reductase; however, positive results for anti-androgenicity can be obtained with compounds that enhance steroid hormone clearance. For the pubertal assays, caution is warranted in interpreting assay results in the presence of significant decreases in terminal body weight. The pubertal assay test guidelines also provide performance criteria that may not be met by some laboratories; the three laboratories in the inter-laboratory validation only partially met these criteria. Thus, when implementing the Tier I mammalian assays, dose selection and performance criteria remain a challenge. However, using both EDSP screening data and previous toxicity data in a "weight of evidence" evaluation will provide the most accurate evaluation of potential endocrine activity.

PS 2376 EFFECTS OF FASTING ON ENDOGENOUS PTH LEVELS IN CYNOMOLGUS MONKEYS.

C. Ruh[1], N. Doyle[1], P. Bednarek[2] and S. Y. Smith[1]. [1]*Charles River Laboratories, Senneville, QC, Canada and* [2]*Cytochroma Inc., Markham, ON, Canada.*

PTH is an important endocrine regulator of calcium and phosphorus and is routinely assessed in studies affecting bone metabolism. The objective of this study was to determine the effects of fasting on endogenous PTH levels in cynomolgus monkeys. Blood samples were taken from 6 monkeys per sex, 1.5 to 3.5 years old, on 4 occasions under fasted or non-fasted conditions and serum PTH levels compared. Samples were analyzed using an ELISA kit (catalogue No. 60-3100, Immutopics). Animals had access to certified commercial primate food twice daily. Blood was sampled in the late afternoon at approximately the same time of day on each occasion with or without a 7-hour pre sampling fasting period. The first set of samples was collected under fasted conditions (Day 1); the second and third under non-fasted conditions (Day 8 and Week 6); and the last sample was collected under fasted conditions (Week 7). Day 1 results were quantifiable for all animals with means of 56.78 pg/mL and 34.20 pg/mL for males and females, respectively. Day 8 results were below the lower level of quantitation (LLOQ = 13.2 pg/mL) for 4/6 males with a mean of 23.15 pg/mL for the remaining 2 individuals, while 4/6 females had results below LLOQ, with a mean of 23.55 pg/mL for the remaining 2

individuals. Week 6 results were quantifiable for 5/6 males with a mean of 46.28 pg/mL, while 4/6 females had quantifiable results with a mean of 43.75 pg/mL. The last samples (Week 7 (fasted)) PTH values were quantifiable for all animals with means of 82.93 pg/mL and 57.38 pg/mL for males and females, respectively. Results below LLOQ were only obtained on the occasions when animals had access to food prior to blood sampling (Day 8 and Week 6), with lower means when compared to Day 1 and Week 7. In conclusion, increased PTH levels following a 7-hour fasting period relative to the unfasted state provided more quantifiable and consistent values for data interpretation. This should be considered when developing a study design involving PTH assessment and, when possible, animals should be fasted prior to sampling.

PS 2377 EFFECTS OF ACUTE AND CHRONIC ALCOHOL EXPOSURE ON CELL SIGNALING IN BETA-ENDORPHIN (βEP) NEURONS OF THE HYPOTHALAMUS.

L. Louis and S. Dipak. *Rutgers University, New Brunswick, NJ.*

The endogenous opioid system is known to play a role in different aspects of alcohol addiction. Specifically, β-endorphin (β-EP) has long been suspected of making a major contribution to the positive reinforcement and motivational properties of alcohol. Several studies have indicated that repeated administration of alcohol significantly attenuate β-EP expression in the hypothalamus and other various limbic areas, while acute administration of ethanol increases β-EP neurotransmission. In the rodent central nervous system, β-EP neurons are primarily located in the arcuate nucleus and the periarcuate area of the medial basal hypothalamus. β-EP produced in neurons in the arcuate area are derived from the pro-opiomelanocortin (POMC) peptide which also gives rise to several other peptides in these β-EP neurons including as alpha melanocyte stimulating hormone and adenocorticotropin hormone. The cellular mechanisms regulating POMC gene expression and subsequent β-EP expression under acute and chronic alcohol treatments have not been well characterized. Using immunohistochemical double staining techniques we demonstrate that changes in β-EP expression are affected by changes in the calcium dependant signaling molecules pCAMKII and its downstream molecules c-FOS and p-CREB after both acute and chronic alcohol treatments. Our findings demonstrate that under acute alcohol treatment β-EP expressing cells increase in the hypothalamus while β-EP producing neurons also increase the calcium dependant signaling molecules pCAMKII, pCREB and CFOS. However under chronic alcohol treatment β-EP expression in the hypothalamus decreases while β-EP neurons also expressing pCAMKII, pCREB and CFOS decrease in the hypothalamus. These results give insight into the possible cell signaling molecules involved in the regulation of β-EP regulation in the arcuate area of the hypothalamus under the influences of acute and chronic alcohol exposure.

PS 2378 MIXTURES OF ANTI-ANDROGENS AT ENVIRONMENTALLY RELEVANT CONCENTRATIONS DISPLAY SIGNIFICANT ADVERSE EFFECTS ON HORMONES AND GENE EXPRESSION COMPARED TO SINGLE EXPOSURES AND THE CONTROL.

J. P. Crago[1] and R. Klaper[2]. [1]*Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI and* [2]*School of Freshwater Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI.* Sponsor: R. Hutz.

Chemicals used by humans can enter into the aquatic environment through a variety of pathways. In some cases these chemicals have been shown to be potent agonists or antagonists of reproductive hormones. Most studies that examined exposures to these compounds look at single exposures and have no adverse effects on the organism at concentrations commonly found in the aquatic environment. There have been few studies that examine the potential additive effects of mixtures at environmentally relevant concentrations, and specifically effects from compounds with the same adverse outcome but with different modes of action. The goal of this study was to assess the effects of anti-androgenic mixtures versus individual anti-androgens on fish at concentrations commonly found in the aquatic environment. In this study male fathead minnows (Pimphales promelas) were exposed to the pesticide linuron (1ppb), di-ethylhexyl phthalate (DEHP) (12ppb), and in a mixture (linuron and DEHP) for a 28-day period. Gene expression profiles of the individual chemicals and chemical mixture were determined using cDNA microarrays followed by qRT-PCR were performed, and plasma testosterone concentrations and gonad-somatic index (GSI) were measured. The results from this study show that adverse effects were chemical dependent. Fish exposed to linuron showed a significantly lower GSI index as compared to control fish, DEHP treated fish, and fish treated to the mixture. Testosterone levels of fish treated with the mixture were significantly lower then that of the control, linuron and DEHP exposed fish. Genes involved in androgen production, such as cytochrome P450 17 and 11a did not differ amongst treatments, but alternate pathways were modified. These results indicate that compounds shown to have anti-androgenic effects can have different adverse outcomes when combined in a mixture.

PS 2379 COMPARATIVE EVALUATION OF THE EFFECTS OF POLYBROMINATED DIPHENYLETHER (DE-71) AND ETHYLENE-BIS-TETRABROMOPHTHALIMDE (EBTBP) ON THYROID HORMONE-MEDIATED ANURAN METAMORPHOSIS.

D. Fort[1], R. Rogers[1], P. Guiney[2] and J. Weeks[2]. [1]*Fort Environmental Labs, Stillwater, OK and* [2]*S.C. Johnson & Sons, Racine, WI.*

An amphibian metamorphosis assay has been proposed by the US EPA in their Tier 1 endocrine disruption screening program (EDSP). Previous studies suggested that the brominated flame retardant mixture, DE-71 impacts the function of the thyroid axis during metamorphosis in Xenopus laevis. Since brominated diphenylether (BDE) flame retardants are currently being replaced by a variety of alternative compounds, we compared the effects of a potential BDE replacement, ethylene-bis-tetrabromophthalimide (EBTBP), and DE-71 on thyroid-mediated metamorphosis. Tier 1 EDSP amphibian metamorphosis assays were conducted exposing stage 51 X. laevis to 0.0, 0.4, 0.9, 2.1, and 3.8 μg/L DE-71 and 0.0, 4.4, 8.8, 17.5, and 35 μg/L EBTBP from stage 51 for 21-d. Results from studies with DE-71 indicated that the rate of metamorphosis decreased with increasing concentration with no marked effect on thyroid gland histology. EBTBP exposure did not alter the rate of metamorphosis or normal histology of the thyroid gland. Serum thyroxine (T4) and triiodothyronine (T3), thyroid gland T4, and thyroid hormone receptor beta (TRβ) expression in tail tissue were measured in stage matched larvae at the conclusion of exposure. Significantly lower levels of both T4 and T3 were measured in serum from DE-71 relative to controls, whereas no effect was observed in the EBTBP-treated larvae. Neither flame retardant significantly altered thyroid gland T4 level, nor altered the expression of TRβ. Thus, although DE-71 slowed metamorphosis by altering peripheral mechanisms of thyroid hormone homeostasis, EBTBP did not alter thyroid-mediated metamorphosis in X. laevis at environmentally relevant concentrations.

PS 2380 OECD VALIDATION STUDY ON THE TRANSFERABILITY, INTRA- AND INTER-LABORATORY REPRODUCIBILITY OF THE ZEBRAFISH EMBRYO TOXICITY TEST.

F. Busquet[1], S. Belanger[2], T. Braunbeck[3], G. Carr[2], M. Halder[1], A. Kleensang[1], A. Lillicrap[4], S. Walter-Rohde[5] and P. Amcoff[6]. [1]*JRC IHCP ECVAM, European Commission, Ispra, Italy,* [2]*Procter & Gamble, Cincinnati, OH,* [3]*University of Heidelberg, Heidelberg, Germany,* [4]*NIVA, Oslo, Norway,* [5]*UBA, Dessau-Rosslau, Germany and* [6]*Environment, Health, and Safety Division, OECD, Paris, France.* Sponsor: M. Embry.

The OECD Acute Fish Toxicity Test Guideline (TG 203) is an integral component in the environmental safety assessment of industrial chemicals, agrochemicals, pharmaceuticals, feed stuffs, and biocides. One of the most promising alternative approaches to the acute fish toxicity test is based on the use of zebrafish embryos. In 2005, the German Federal Environment Agency submitted the draft TG on "Fish embryo toxicity (FET) test" to the OECD Test Guideline Program and a supportive Background Paper. Subsequently, OECD established the ad hoc Expert Group on the Fish Embryo Toxicity Test. Based on the outcome of expert meetings, OECD decided to perform a validation study (coordinated by ECVAM and steered by a validation management group). The validation study aims to evaluate the transferability, and the intra/interlaboratory reproducibility of the Zebrafish FET (ZFET). Newly fertilised zebrafish eggs are exposed for up to 96h to chemicals. 4 apical endpoints are recorded daily as indicators of acute lethality in fish: coagulation of the egg, lack of somite formation, non-detachment of the tail bud from the yolk sac and lack of heart-beat. LC50 values are calculated for 48h and 96h exposure. 7 chemicals are tested at 5 different concentrations in 3 independent runs in at least 4 laboratories with appropriate controls. Stock solutions and test concentrations of 1 laboratory are confirmed. The poster will give an overview on the validation study design, the results, next steps and the correlation of the ZFET with acute fish LC50 data.
"Disclaimer: The opinions expressed and the arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries'"

PS 2381 HISTOPATHOLOGICAL AND ENZYME CHANGES IN *CLARIAS GARIEPINUS* (BURCHELL 1822) EXPOSED TO NITRITE AT DIFFERENT WATER TEMPERATURES.

F. Ajani[1], B. O. Emikpe[2] and O. K. Adeyemo[3]. [1]*Animal Science & Fisheries Management, Bowen University, Iwo, Iwo, Osun, Nigeria,* [2]*Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria and* [3]*Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Nigeria.*

Nitrite is a natural component of the nitrogen cycle in ecosystems. It is an intermediate in the oxidation of ammonium to nitrate. The elevation of ambient nitrite concentration is a potential problem for freshwater fish. This study was designed to investigate the effect of different water temperatures on the toxic effect of nitrite in a freshwater fish. Sixty Clarias gariepinus (300 ± 1.30g), were exposed to nitrite at different water temperatures (27°C and 35°C) for 48hours. Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and total protein levels were assayed in the gill, liver and tissue (skin) of the fish. The statistical analysis was performed using the Statistical Analysis (SPSS 11.0 for Windows). Statistical differences were determined by one-way analysis of variance (ANOVA) and paired-sample t test. ALT and ALP increased significantly ($P<0.05$) in nitrite-intoxicated fish at 35°C compared to the value obtained at 27°C in the organs while a significant decrease ($P<0.05$) was observed for the enzyme AST at 35°C compared to 27°C. Protein level in all the tissues showed a significant decrease in nitrite-intoxicated fish at higher temperature. The histopathological changes observed in the gills of nitrite-treated fish at 35°C were that of congestion and vacuolization while the liver showed generalized fatty degeneration, congestion of central veins and multifocal necrosis. Moderate hydropic degeneration of the epidermal layer was observed in the skin tissue. These results revealed that high temperature can increase the toxic action of nitrite in fish.

PS 2382 CHEMICAL ACTIVATION OF THE NUCLEAR RECEPTOR HR96 IN *DAPHNIA PULEX* BY ENVIRONMENTAL CHEMICALS.

E. Karimullina[1, 3], Y. Li[1] and W. S. Baldwin[1, 2]. [1]*Environmental Toxicology Program, Clemson University, Clemson, SC,* [2]*Biological Sciences, Clemson University, Clemson, SC and* [3]*Institute of Plant & Animal Ecology, Russian Academy of Sciences, Ural Branch, Yekaterinburg, Russian Federation.*

Daphnia pulex is the first crustacean to have its genome sequenced. This genome project provides new insight and data into how an aquatic crustacean may acclimatize or adapt to environmental stressors, including toxicants. We cloned DappuHR96, a nuclear receptor orthologous to the CAR/PXR/VDR group of nuclear receptors. In *Drosophila melanogaster*, HR96 responds to phenobarbital exposure and therefore has been hypothesized to be a promiscuous toxicant receptor. We set up a transactivation assay to determine if *Daphnia pulex* HR96 (DappuHR96) is activated by environmental chemicals. A Gal4 chimeric receptor was made with the ligand binding domain of HR96 fused to the DBD of Gal4. HepG2 cells were cotransfected with HR96 and a luciferase reporter construct. Initial transactivation assays indicate that similar to CAR, HR96 is constitutively active making it difficult to measure highly efficacious activation. However, we found several diverse chemicals that activated HR96, including chlorpyrifos, atrazine, methane arsonate, pyriproxyfen, and estradiol. We also observed that three chemicals, triclosan, androstanol, and fluoxetine, repressed HR96 activity. It is interesting that androstanol represses HR96 activity in CAR and the related receptor HR96; although triclosan was by far the most efficacious inverse agonist. In summary, data indicates that HR96 is a promiscuous nuclear receptor that may act to protect *Daphnia* from aquatic toxicants. Future work will investigate the potential of inverse agonists to improve the sensitivity of our transactivation assay, and produce dose-response curves for some chemical activators. This work is supported by CUCI and the Fulbright Foundation.

PS 2383 SIMULTANEOUS QUANTITATION OF TESTOSTERONE AND ESTRADIOL IN HUMAN CELL LINE (H295R) BY LIQUID CHROMATOGRAPHY-POSITIVE ATMOSPHERIC PRESSURE PHOTOIONIZATION TANDEM MASS SPECTROMETRY.

F. Zhang, D. L. Rick, L. H. Lynn, A. W. Perala, D. R. Geter and M. J. Bartels. *The Dow Chemical Company, Midland, MI.*

The possible interaction of environmental contaminants with the endocrine system has been an environmental issue since the early 1990s. To examine these interactions test guidelines have been introduced by regulatory agencies to screen for possible endocrine active compounds. One of these guidelines is EPA's OPPTS 890.1550 [Steroidogenesis (Human Cell Line-H295R)]. This guideline requires quantifying two major biomarkers (testosterone and estradiol) in various biological test systems. Traditional quantitation methodologies such as Radio Immuno Assay (RIA) and Enzyme-linked Immunosorbent Assay (ELISA) have been used for quantifying low level of steroids; however, those methodologies have drawbacks such as the radioactive safety, antibody availability, separate assay for each biomarker, and lack of selectivity. In the current study, a rapid and sensitive liquid chromatography-positive atmospheric pressure photoionization tandem mass spectrometry method (LC/APPI/MS-MS) has been developed and validated for the simultaneous quantitation of testosterone and estradiol in the H295R cell line. Briefly, the media from cultured cells was extracted with CH2Cl2 containing internal standards of both testosterone-d3 and estradiol-d3, then, the extracted organic layer was concentrated down to dryness. The final residue was derivatized with dansyl chloride solution, and directly analyzed by LC/APPI/MS-MS. The calibration curves, with concentration ranging from 10 to 2500 pg/mL were linear with coefficient >0.99. The lower limits of quantitation for both testosterone and estradiol are 10 pg/mL. This method was successfully validated to support requirements of the current EPA Steroidogenesis guideline and shown to be as sensitive and much less variable than the traditional antibody-based assay methods. This type of method may also provide usefulness for rapid and precise measurements of these two hormones in other in vitro or in vivo test systems.

PS 2384 EFFECTS OF PHARMACEUTICALS USED FOR BREAST CANCER TREATMENT ON REPRODUCTION AND AROMATASE ACTIVITY IN A MARINE FISH.

L. J. Mills[1], R. Gobell[1], G. Zaroogian[1], M. Simoneau[1], D. Borsay Horowitz[1], M. Hotchkiss[2], N. Tinfo[2] and S. Laws[2]. [1]*NHEERL, Atlantic Ecology Division, U.S. EPA, Narragansett, RI and* [2]*NHEERL, Toxicity Assessment Division, U.S. EPA, Research Triangle Park, NC.*

Laboratory experiments were conducted with the marine fish cunner (*Tautogolabrus adspersus*) to evaluate whether four pharmaceuticals used in breast cancer treatment have an impact on reproduction or aromatase activity. Tamoxifen binds to estrogen receptors, while anastrozole, letrozole and exemestane are all aromatase inhibitors. Aromatase, a key enzyme in estrogen biosynthesis, is critical to normal reproduction in fish, just as in mammals. Spawning cunner were treated with tamoxifen (0, 0.5 or 5 mg/kg) or one of the three aromatase inhibitors (0, 75 or 750 μg/kg) administered by oral gavage on days 0, 4, 8, 12 and 16 of each experiment. Egg production, viability and fertility were determined daily. On day 17, gonadosomatic index (GSI) was determined, and brain and ovary tissues were flash-frozen for later analysis of aromatase activity. In tamoxifen-treated fish, egg production was significantly reduced, but downward trends in egg fertility and viability were not significant. Ovarian aromatase activity was significantly increased in fish treated with 5 mg/kg tamoxifen. Of the aromatase inhibitors, anastrozole and letrozole significantly decreased egg production in fish treated with the high concentration (750 μg/kg), while exemestane had no effect. There was no effect on egg fertility or viability in fish treated with any aromatase inhibitor. Female GSI was significantly reduced in the anastrozole treatments, significantly increased in 75 μg/kg letrozole treatment, and unaffected by exemestane treatment. Effects on aromatase activity were variable depending on treatment and tissue (brain or ovary). For example, in fish treated with anastrozole, aromatase activity was significantly decreased in brains, while activity was increased in ovaries. Results indicate tamoxifen and aromatase inhibitors can impact fish reproduction. The effect of these pharmaceuticals on aromatase activity appears to be chemical- and tissue-specific.

PS 2385 THE APPLICATION OF THERMOLUMINESCENCE IN ECOTOXICOLOGY.

G. Repetto[1], J. L. Zurita[2], M. Roncel[3] and J. Ortega[3]. [1]*Biología Molecular e Ing. Bioquímica, University Pablo de Olavide, Sevilla, Spain,* [2]*Fundación Progreso y Salud, Sevilla, Spain and* [3]*Instituto de Bioquímica Vegetal y Fotosíntesis. University of Sevilla-CSIC, Sevilla, Spain.* Sponsor: M. Lanaspa.

Thermoluminescence consists of illuminating the sample at a temperature low enough to block charge recombination, and revealing the different types of charge pairs as thermoluminescence emission bands by progressive warming. In photosynthetic organisms, most of the thermoluminescence emissions are due to the reverse reaction of charge separation, induced by the light in the photosystem II. Illumination of photosynthetic material at low temperature produces a stable separation of charges among the donor and the acceptor of photosystem II. The different recombinations generate characteristic thermoluminescence bands. A model was developed to evaluate the alterations produced by environmental chemicals on the physiology of photosystem II thanks to standard thermoluminescence. In addition, high temperature thermoluminescence was applied, quantifying HTL2 band as a biomarker of lipid peroxidation on vegetables. The effects of the chemicals bromobenzene, diethanolamine, chloroquine, sodium fluoroacetate, propyl gallate and indium nitrate were studied in the alga Chlorella vulgaris. The most relevant effects were observed for diethanolamine and chloroquine, the least and most potent inhibitors of Chlorella proliferation, respectively. A considerable reduction on the

functionality of photosystem II and an increase on lipid peroxidation were detected. Furthermore, the study of thermoluminescence emissions is a useful technique to study the effects of environmental chemicals on photosynthetic organisms.

PS 2386 TISSUE DISTRIBUTION OF MERCURY IN ALASKA SCULPIN SPECIES.

S. Bhojwani, C. Lieske and T. O'Hara. *University of Alaska Fairbanks, Fairbanks, AK.*

Mercury contamination of marine species is a big concern for subsistence hunters as well as fish consumers today. Mercury accumulates in marine animals and is associated with many negative health impacts including neurotoxicity. For this study, select tissue samples from nineteen subsistence-caught sculpin species were taken, freeze dried, and analyzed for total mercury (THg) via a DMA-80 (Direct Mercury Analyzer-80) in order to determine the differences in mercury within and across different tissue types (liver, muscle, heart and kidney). Additionally, differences in mercury deposition within skeletal muscles were assessed. Wet weight THg concentrations ranged from 0.58 to 0.935 ppm in the skeletal muscle, 0.015 to 0.39 ppm in the liver, 0.017 to 0.24 ppm in the kidney, and from 0.016 to 0.17 ppm in the heart. It was noted that as fish size increased, the THg present in the fish likewise increased. Significant differences were noted between internal organs and muscle tissue, with THg concentrations in muscle greater than THg in the internal organs (liver, heart and kidney). A significant difference between the mean mercury content across the four muscle samples was detected, with the mean mercury at the caudal end greater than the mean mercury at the most cranial end for 18 of 19 fish. However, the observed difference may not be biologically significant due to the heterogeneity of the muscle samples. These data suggest that the internal organs of these sculpin species have much less mercury content in comparison to the muscle tissue, and that fish that are small or of average size may be safer to consume than those of larger sizes.

PS 2387 PROTEOMIC ANALYSIS OF A MODEL FISH SPECIES EXPOSED TO INDIVIDUAL PESTICIDES AND A BINARY MIXTURE.

A. D. Biales, D. C. Bencic, K. Blocksom, D. L. Lattier, R. Flick and L. Jim. *ORD NERL, U.S. EPA, Cincinnati, OH.* Sponsor: H. Choudhury.

Pesticides are nearly ubiquitous in surface waters of the United States, where they often are found as mixtures. The molecular mechanisms underlying the toxic effects of sub-lethal exposure to pesticides as both individual and mixtures are unclear. The current work aims to identify and compare differentially expressed proteins in brains of male fathead minnows (Pimephales promelas) exposed for 72 h to permethrin (7.5 μg/L), terbufos (57.5 μg/L) and a binary mixture of both. Twenty-four proteins were found to be differentially expressed among all three treatments relative to the control using an ANOVA followed by a Dunnett's post-hoc test ($p \leq 0.05$). One protein was found to be differentially expressed among all treatment groups and one protein was in common between the terbufos and the mixture group. Fifteen spots were successfully sequenced using LC-MS/MS sequencing. Proteins associated with the ubiquitin-proteasome system, glycolysis, the cytoskeleton and hypoxia were enriched. As a second objective, we attempted to establish protein expression signatures (PES) for individual permethrin and terbufos exposures. We were unable to generate a useable PES for terbufos; however, the permethrin PES was able to distinguish between control and permethrin-exposed individuals in an independent experiment with an accuracy of 87.5%. This PES also accurately classified permethrin exposed individuals when the exposure occurred as part of a mixture. The identification of proteins differentially expressed as a result of pesticide exposure represent a step forward in the understanding of mechanisms of toxicity of permethrin and terbufos. They also allow a comparison of molecular responses of the binary mixture to single exposures. The permethrin PES is the first step in establishing a method to determine exposures in real-world scenarios.

PS 2388 TRIBUTYLTIN (TBT), AN ENDOCRINE DISRUPTOR IN ZEBRAFISH.

C. L. McGinnis and J. F. Crivello. *Physiology and Neurobiology, University of Connecticut, Storrs, CT.*

Tributyltin (TBT) is an antifouling agent that has been implicated in the masculinization of fish species worldwide; however, its masculinizing mechanism is not fully understood. We have examined the actions of TBT as an endocrine disruptor on three different endpoints in zebrafish (Danio rerio): 1) directly on nuclear receptors in HeLa cells, 2) on enzymatic activities of hepatic acylated and sulfated testosterone, and 3) on the expression of androgenic and estrogenic developmental markers in vivo. Zebrafish estrogen receptor (zfERα, zfERβ1 and zfERβ2) dependent activity was inhibited by TBT in a dose dependent manner, with an ED50 between 5nM and 50nM. TBT had no effect on zebrafish androgen receptor (zfAR); however, TBT inhibited zebrafish aromatase (zfCYP19b-luc) dependent activity in a dose dependent manner. In HeLa cells, TBT elicited a dose dependent activation of the peroxisome proliferator-activated receptor gamma (PPARγ) and retinoid X receptor alpha (RXRα) heterodimer, mediated through RXRα. In zebrafish exposed to 1 to 5mg/kg TBT, there was increased liver sulfotransferase activity and decreased acyl-CoA testosterone acyltransferase activity. Real-time PCR analysis of sexual differentiation markers in zebrafish exposed to TBT revealed tissue-specific responses. TBT overall has a masculinizing effect, but the masculinizing effect may be tempered by the metabolism of TBT to dibutyltin (DBT).

PS 2389 THE EFFECTS OF COPPER PYRITHIONE ON DEVELOPING ZEBRAFISH EMBRYOS.

K. M. Almond and L. D. Trombetta. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Copper pyrithione, an active component in antifouling biocides predominantly found in marine paints, has been shown to cause anomalies during early development of mummichogs (Fundulus heteroclitus). Since a strict regulatory use of organotins as antifouling agents has been imposed, a frequent substitute by foreign markets has been the use of metal pyrithiones, principally zinc and copper. Zebrafish (Danio rerio) embryos were exposed within the first hour after fertilization to increasing concentrations of copper pyrithione (2, 4, 8, 12, 16, 32 and 64 μg/l) for 24 hours. Morphological abnormalities at 30, 60, and 120 hours post fertilization were recorded. Hatchability was measured at every 12 hours for 5 days. Following the 24 hour exposure, results show morphological abnormalities, particularly of the notochord at concentrations of 12 μg/l and higher. The notochord was severely twisted and abnormally segmented as the concentrations increased. Distortions of the notochord began in the tail at the lower concentrations and proceeded rostrally as doses increased. Edema was observed in the cardiac and yolk sac regions at 12 and 16 μg/l concentrations. Hatching rate decreased in a dose dependent manner. At 120 hours post fertilization, 47 percent of embryos hatched, while all of the controls hatched by 96 hours post fertilization. Copper pyrithione has shown to be toxic to zebrafish at concentrations of 12 μg/l and higher. The abnormalities and deformities observed in fish larvae would significantly decrease their chance of survival in non-controlled environments.

PS 2390 TISSUE SPECIFIC EXPRESSION OF ORGANIC ANION TRANSPORTING POLYPEPTIDES IN NATIVE FISH SPECIES.

K. Steiner[1], K. Shigehiro[2], A. Meyer[2] and D. R. Dietrich[1]. [1]*Human & Environmental Toxicology, University of Konstanz, Konstanz, Germany and* [2]*Zoology and Evolutionary Biology, University of Konstanz, Konstanz, BW, Germany.*

Microcystins (MCs) are naturally occurring toxins that are produced by many species of cyanobacteria. MCs are cyclic heptapeptide cyanotoxins with >80 structural variants and a molecular weight ranging from 900 to 1100 Da. Toxic cyanobacterial blooms in aquatic ecosystems have been associated with dramatic changes in species distribution. It is hypothesized that the bioavailability of MCs, via specific MC transporting organic anion transporting polypeptides (Oatps), governs the susceptibility of different species and life-stages and thus the downstream adverse effects on organisms and ecosystems more strongly than the level of MC concentrations alone in the ambient environment. Consequently the presence of Oatps and their organ specific expression in fish may be key to understanding the biological effects of MCs. Recently verified short Oatps from native fish species were sequenced using RACE PCR and phylogenetically analyzed in order to identify specific Oatp subtypes, which were used for designing gene specific primers. The improved primers were employed to analyze the Oatp subtype expression profile in different fish tissues via real-time quantitative PCR. The different sequences obtained were verified as Oatp by BLAST and elongated using RACE PCR. A sequence identified as Oatp1d1 was expressed in a tissue-dependent manner, suggesting an organ-specific transport of MC.

PS 2391 INTERACTIVE EFFECTS OF BENZO(α)PYRENE, BENZALKONIUM CHLORIDE, AND SEWAGE EFFLUENT ON MEDAKA EMBRYOS.

A. McElroy and J. Gondek. *School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY.* Sponsor: R. Di Giulio.

Chemical mixtures represent a common stressor to organisms, yet traditional toxicological approaches rarely examine interactive effects of contaminants found in the environment. For aquatic organisms, particularly those living in urban watersheds, exposure to mixtures from sewage inputs, surface run-off, and contaminated sediments is a chronic problem. We evaluated the individual and combined effects of

model compounds from three groups of ubiquitous environmental contaminants: quaternary ammonium compounds (QACs) used as disinfectants, polycyclic aromatic hydrocarbons (PAHs) released through use of fossil fuels, and sewage effluent, on embryos of the Japanese Medaka (*bital*Orizias latipes*eital*), using benzalkonium chloride (BAC), a high production volume chemical, as a model QAC, and benzo(α)pyrene (BaP) as a model PAH. We evaluated inhibition of multixenobiotic resistance (MXR) efflux pumps by quantifying accumulation of calcein-AM, and developmental toxicity by visually scoring developmental deficits and death. BAC inhibited MXR pump activity in a dose dependent manner, while BaP had no effect. Treated sewage effluent also inhibited pump activity, although adding BaP to effluent abolished the effect. BAC, BaP, and treated sewage effluent caused cranial facial, skeletal and heart deformities, as well as cardiac edema, all at sublethal concentrations. Co-exposure to BAC and BaP lead to greater than additive effects, while co-exposure to BaP had no significant effect on effluent toxicity. That BAC and sewage effluent are potent inhibitors of MXR pumps indicates exposure to these compounds can compromise this important defense system that limits cellular exposure to moderately hydrophobic contaminants or contaminant metabolites. The ability of these compounds to act as developmental toxicants is also noteworthy as is the enhanced toxicity observed with BaP exposure.

PS 2392 THE GULF OIL CRISIS: IMPACT ON WHALES.

S. S. Wise[1, 2, 3], I. Kerr[2, 4], J. P. Wise[1, 2, 4], C. F. Wise[1, 2, 4], J. Wise[1, 2, 4], C. Gianios[1, 2, 4], M. Braun[1, 2, 4], B. Wallace[4], I. Glass[4], R. Walker[4], C. LaCerte[1, 2, 4], J. McKay[1, 2, 4], T. Li Chen[1, 2, 4], J. Martino[1, 2, 4], G. Chapman[1, 2, 4], R. Duffy[1, 2, 4], R. Leighton[1, 2, 4], K. Joyce[1, 2, 4] and J. P. Wise[1, 2, 3]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME, [2]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME, [3]Department of Applied Medical Science, University of Southern Maine, Portland, ME and [4]Ocean Alliance, Lincoln, MA.*

The recent disaster in the Gulf of Mexico resulted in the release of 4.9 million barrels of oil and 2 million gallons of chemical dispersants. These chemicals were used in unprecedented ways and amounts and the toxicity of them is poorly understood. The impact of the oil and dispersants on wildlife in the gulf is of significant concern. Many animals, including whales, fish and turtles, use the Gulf of Mexico as calving and spawning grounds. Of particular concern are whales as they are a key indicator species for the health of our oceans. Two resident populations include sperm whales and Bryde's whales which are at particular risk because their population size is small. To address this crisis, we launched a voyage to assess the impacts of these pollutants on whale health. The research vessel Odyssey, a 93 foot ketch equipped with state-of-the art whale tracking, sampling equipment and cell culture facilities, was used for the voyage. Our scientific approach assessed 1) acute toxicity based on visual, acoustic and behavioral data, 2) subacute and chronic toxicity by measuring contaminant levels in whale biopsies, and 3) chronic toxicity by dosing cultured cells from these animals with petroleum products, dispersants and metals. In doing this early assessment a baseline set of toxicological data was established to compare it to similar efforts in the Atlantic as well as future years. The Voyage was supported by generous donations from the University of Southern Maine, The Campbell Foundation, The Ocean Foundation, The Marisla Foundation and Quiznos among many other individual donors and supporters of the Wise Laboratory and Ocean Alliance.

PS 2393 IMPOSEX IN *PLICOPURPURA PANSA (MOLLUSCA:NEOGASTROPODA)* AS A POTENTIAL MARINE POLLUTION BIOINDICATOR IN NAYARIT AND SINALOA, MEXICO.

D. Domínguez[1], A. Patron[2, 1], L. Robledo[1], J. Velazquez[1] and B. Quintanilla[3, 1]. *[1]Laboratorio de Toxicologia, Universidad Autónoma de Nayarit, Tepic, Nayarit, Mexico, [2]Fisiologia, Universidad Autonoma de Mexico, DF, Mexico and [3]Toxicologia, Cinvestav, DF, Mexico.*

Imposex is a biomarker of marine species such as gastropods for the presence of a penis in females, and it has been used worldwide as a biomarker of exposure to organotin compounds (OTs). Samples of Plicopurpura pansa were collected and examined to assess the sex ratio, the presence of females with imposex and lipid peroxidation. These populations were obtained from habitats exhibiting three presuming different levels of pollution (taking into account their proximity to pollutant places due to human activity) in Mexico such as Isabel Island and Santa Cruz, in Nayarit State, as well as Olas Altas, in Sinaloa State. Imposex in female mollusks was scored as the presence of a pseudopenis. Females and males were classified as those having normal characteristics. Lipid peroxidation was determined by the Thiobarbituric acid reactive species. The sex ratio of male:female in Olas Altas and Santa Cruz were 2:1 in both cases, whereas the sex ratio in Isabel Island was 1:1. The presence of imposex was 17.24% in Olas Altas, followed by 11.24% in Santa Cruz and 2.3% in Isabel Island. The lipoperoxidative damage was greater in organisms from Olas Altas, followed by Santa Cruz and Isabel Island. According to these results, the more proximal to pollutant areas, the more oxidated the snails are, and the more number of imposex cases.

PS 2394 *IN VITRO* METABOLISM OF DIAZEPAM IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*).

C. Overturf and D. Huggett. *University of North Texas, Denton, TX.*

Many human pharmaceuticals have been detected in surface waters where they may bioconcentrate and pose adverse effects to aquatic life. Thus, the following study examined the *in vitro* metabolism of diazepam, a model benzodiazepine, in microsomal fractions of the liver from channel catfish to better determine its bioconcentration potential. In mammals, diazepam is metabolized to the active intermediates nordiazepam and temazepam via CYP2C19 and CYP3A4/5, respectively. Both intermediates are then further metabolized via CYP3A4 to the active metabolite oxazepam. This study attempted to investigate the loss of parent compound (diazepam), as well as the generation of active metabolites. Briefly, livers from catfish (n=3) were harvested and microsomal fractions were prepared. An NADPH regeneration system was used to simulate phase I reactions and 1000uM diazepam was added to the system. Samples were taken over a ninety-minute time period and placed in methanol to stop the reaction. The samples were then processed and analyzed using LC-MS/MS. A significant loss (14%) of parent compound was measured within forty-five minutes. Over the same ninety-minute period, both nordiazepam and temazepam were detected within fifteen minutes. Their formation plateaued within thirty minutes at 35 parts per billion (ppb) and 102 ppb, respectively. Interestingly, oxazepam was not detected during the ninety-minute incubation period. This study shows that microsomal fractions of channel catfish liver contain the necessary enzymes for the metabolism of diazepam into two pharmacologically active intermediates. However, the time period may not have been sufficient for the generation of oxazepam to occur, or catfish enzyme systems may be unable to generate this secondary metabolite. Further studies will attempt to elucidate the metabolic pathway of diazepam metabolism and active metabolite formation in channel catfish.

PS 2395 QUEEN CONCH (STROMBUS GIGAS) REPRODUCTIVE DYSFUNCTION IN NEARSHORE AREAS OF THE FLORIDA KEYS – A POSSIBLE LINK TO CU AND ZN.

D. Spade[1], A. Feswick[1], R. A. Glazer[2], D. S. Barber[1] and N. D. Denslow[1]. *[1]Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL and [2]Fish and Wildlife Research Institute, Florida Fish and Wildlife Conservation Commission, Marathon, FL.*

In the Florida Keys, queen conchs (Strombus gigas) residing offshore (OS) develop gonads and are capable of reproduction, while near-shore (NS) conchs lack sufficient gonadal development for reproduction. A custom queen conch microarray (GEO accession GPL8934) was used to study differences in gene expression between OS and NS conch. 257 transcripts were differentially regulated in testis, 1368 in ovary, and 1153 in the digestive gland of females (ANOVA, $p<0.01$, FDR=5%). Functional enrichment analysis based on Gene Ontology (GO) terms (Fisher's exact test, $p<0.05$) indicated that spermatogenesis, small GTPase-mediated signal transduction, proton transport, and mitochondrial transport were affected in the testis; oogenesis, germ cell development, and translation in the ovary; and cation transport and lipid metabolic processes in the digestive gland of females. Inductively coupled plasma mass-spectrometry (ICP-MS) identified elevated Cu and Zn in NS conch tissues, including Zn in the digestive gland and possibly Cu in the digestive gland and gonad. Metal-responsive genes present in the microarray data, including the Zn transporter SLC30A5 (down-regulated in NS digestive gland) and a putative metallothionein (up-regulated in NS ovary) further implicate Zn and Cu as potential stressors involved in reproductive dysfunction of NS conchs. In vivo exposures with Cu and Zn are ongoing to determine whether these two metals produce effects similar to those observed in wild conchs.

PS 2396 COAL MINING INDUCES OXIDATIVE STRESS, DNA DAMAGE, AND ALTERATIONS IN RED BLOOD CELL MORPHOLOGY IN WILD MICE.

A. Guerrero-Castilla[1], J. Olivero-Verbel[1], M. Cabarcas-Montalvo[1], H. Corrales-Aldana[1], M. Guerra-Hernandez[1], L. Carranza-Lopez[1] and M. Moreno-Contreras[2]. *[1]Environmental and Computational Chemistry Group, University of Cartagena, Cartagena, Colombia and [2]Popular University of Cesar, Valledupar, Colombia.*

Coal mining is one of the most important economical activities in Colombia. However, its environmental and human impact has been neglected. The aim of this study was to determine the status of different molecular and cellular markers in

wild mice (Mus musculus) from areas with coal mining operations, comparing them to non-exposed animals. Mice were captured from houses around coal mines in the Cesar department of Colombia, and blood and tissue samples were collected. RT-PCR was used to evaluate liver expression of selected genes. DNA damage was assessed by measuring the extent of DNA migration in peripheral blood leukocytes using comet assay, and Wright-stained blood smears were prepared for comparative morphological assessment of blood cells. Compared to control group, liver mRNA metallothionein I and II (up to 370 fold), as well as Nqo1 (up to 4 fold) were up-regulated, whereas Dmt1 and Gpx3 were donwregulated. This profile suggests mice are being exposed to heavy metal pollution and this may be altering the oxidative-stress status of these organisms. Small increases in Cyp1a1 expression were observed as well, probably from PAHs exposure. Other genes such as Ccnd1, Ddit3 and SOD did not show any differences in expression between groups. Comet assays revealed that DNAs from mice obtained near coal mines are significantly more damaged than those from control animals. Exposed mice also showed relative augmented polychromatophilia and microcyte counts. Taken together, these results suggest that coal mining activities releases environmental pollutants that impact the biota, inducing expression changes on genes related to oxidative stress, as well as DNA damage and alterations in the morphology of red blood cells. Accordingly, preventive measurements should be taken to reduce human and environmental health risks. University of Cartagena-Colciencias (110749326186) and Department of Cesar (1235).

PS 2397 LEAD POISONING FROM INGESTED AMMUNITION IS PRECLUDING RECOVERY OF THE ENDANGERED CALIFORNIA CONDOR.

M. Finkelstein[1], D. Doak[2], J. Grantham[4], D. George[3] and D. R. Smith[1]. *[1]University of California, Santa Cruz, CA, [2]University of Wyoming, Laramie, WY, [3]NPS, Pinnacles National Monument, CA and [4]U.S. Fish and Wildlife Service, Ventura, CA.*

Lead poisoning of endangered California Condors (Gymnogyps californianus) is a primary factor limiting the survival and recovery of this decimated species that was reduced to 22 wild individuals by 1982. Despite substantial management efforts to reduce lead exposure risk, California condors continue to be lead poisoned on a regular basis. For example, in 2009 almost 50% of the condors monitored at one California release site (Pinnacles National Monument) received clinical intervention (chelation therapy) for lead poisoning. Although lead ammunition is considered a major source of lead poisoning to condors, direct evidence is limited. We used lead isotopic analysis of blood samples collected from pre-release (n=17) and free-flying condors in California (n =70) and a representative selection of lead-based ammunition (n=71) to evaluate the likelihood that condors are exposed to a lead source consistent with that observed in lead ammunition. Isotopic analysis demonstrated that the lead isotopic signature of free-flying condors is different than the lead isotopic signature of pre-release birds. Furthermore, using an isotopic mixing model, we found that ~90% of blood samples from free-flying condors had an isotopic composition that is best explained by exposure to lead-based ammunition. To evaluate the sub-lethal health impacts of lead exposure in condors, we measured aminolevulinic acid dehydratase (ALAD) activity in condor blood samples (n = 60), and found that ALAD activity was inhibited by >60% at blood lead levels >20 μg/dL. Out of 760 condor blood samples collected between 2004-2009 in California, ~35% had blood lead ≥20 μg/dL, suggesting that a large percentage of free-flying condors are chronically exposed to lead levels well-know to produce morbidity. Our work demonstrates that ammunition is the principal source of lead poisoning to California condors and ~ 1/3 of free-flying condors are experiencing toxicological effects from lead.

PS 2398 OCCURRENCE OF SSRI'S IN PUGET SOUND STREAMS AND EFFECTS ON FISH: AN INTEGRATED FIELD AND LAB STUDY.

I. Schultz[1], E. Walters[2], M. Sroufe[3] and J. Nagler[4]. *[1]Battelle PND, Sequim, WA, [2]Battelle PND, Sequim, WA, [3]Battelle PND, Sequim, WA and [4]Biological Sciences, University of Idaho, Moscow, ID.*

There are increasing concerns over environmental impacts of pharmaceuticals released into surface waters from municipal wastewater discharges. In this study, we initially monitored four different streams in the greater Puget Sound region for the occurrence of estrogenic contaminants and select pharmaceuticals. This initial survey identified one site (White River, WA) where estrogens (E2 and E1) were occasionally observed while two SSRIs, fluoxetine and sertraline were always observed in both grab samples of river water and passive sampling devices deployed for several weeks at the site. In some samples, the concentration of the SSRIs exceeded 30 ng/L. This surprising finding prompted us to do additional experiments to better understand the toxicokinetics and disposition of SSRIs in fish and assess whether exposure to either fluoxetine or sertraline impacted fish reproduction. During our initial survey season, we placed caged, sexually mature rainbow trout at each site for up to eight weeks and then subsequently spawned them to assess fertility. The only site where we observed poor fertility was at the White River site. Subsequent laboratory studies exposed breeding pairs of fathead minnows to water collected from the White River site (near a sewage outfall) and controlled exposures to sertraline. Toxicokinetic studies of fluoxetine in trout provided interesting results in that plasma concentrations were nearly undetectable during three week exposures to 1 ug/L, yet brain levels were relatively high as was concentrations in the liver and kidney. These latter findings would be consistent with active sequestration of SSRIs in these tissues. Our collective findings indicate that fish can rapidly absorb SSRIs and concentrate them in target tissues such as the brain causing neuroendocrine changes that are associated with decreased reproduction.

PS 2399 *DROSOPHILA* AS A MODEL TO DISSECT THE SIGNALING PATHWAY MEDIATING THE TOXICITY OF FUNGAL VOLATILE ORGANIC COMPOUNDS IN DOPAMINERGIC NEURONS.

A. A. Inamdar and J. W. Bennett. *Rutgers University, New Brunswick, NJ.* Sponsor: J. Richardson.

Epidemiological studies link exposure of fungi with building related illness commonly called as "Sick Building Syndrome" (SBS). SBS is a constellation of symptoms such as malaise, lethargy, memory loss, difficulty in concentration along with organ specific symptoms. The majority of research has correlated the role of mycotoxins and spores from fungi to the SBS. We pioneer to investigate the role of volatile organic compounds (VOCs) emitted by fungi to the organ specific symptoms of SBS usning reductionist approach. Our recent findings suggest that upon exposure of lower concentration of certain fungal VOCs are capable of cause truncation of life span, movement disorders and dopaminergic neurons in *Drosophila*. We further used *Drosophila*, inexpensive genetic model to further identify the candidate signaling genes mediating the toxicity of fungal volatile organic compounds. We used 1-octen-3-ol, C8 compound ubiquitously expressed by almost all the fungi. This compound was exposed to *Drosophila* loss of function mutants for JNK, Akt and MAPK signaling pathway genes. In addition, transgenic lines overexpressing JNK, Akt and MAPK in dopaminergic neurons were also tested against 1-octen-3-ol. So far, our results suggest that Akt signaling pathway is crucial for counteracting the toxicity of fungal VOC, 1-octen-3-ol in dopaminergic neurons. We further will investigate other fungal VOCs to dissect the crucial signaling pathway genes mediating the toxicity of fungal VOCs.

PS 2400 COMPARATIVE RESPONSES TO DIPHENHYDRAMINE IN TWO FISH MODELS.

J. Berninger, B. W. Brooks and B. Du. *Baylor University, Waco, TX.*

Diphenhydramine (DPHM) is an antihistamine with multiple modes of action (MOA) used to treat a variety of symptoms, including rhinitis and, at higher doses, sleeplessness. Because of its widespread use, DPHM has been detected in the water, sediments, and fish residing in streams receiving discharges of treated wastewater effluent (< 1 μg/L). Consistent with observations of other therapeutics, at environmental exposure scenarios, acute toxicity to fish is not likely in the developed world (Pimephales promelas, fathead minnow LC50 = 2.18 mg/L). Though the chronic effects elicited by pharmacological MOAs are not well understood in fish, they are well developed in mammals. In this study, we tested pharmacokinetic models using a mammal to fish "read across" approach, which suggested that antihistamine MOA related responses may occur at water exposures of ~ 1 μg/L, whereas narcotic effects were predicted to occur at higher concentrations. The evolutionary conservation of drug targets suggests that narcotic MOAs should be similar in fish and mammals. The antihistamine MOA is likely to only occur in the more evolutionarily advanced Perciform fishes, where histamine is released as part of a mast cell degranulation immune response, which is important for anti-parasitic and anti-infective pathways. To test the narcotic effects of DPHM fathead minnows were subjected to two types of studies. The first study design included a standard 7 day larval chronic toxicity test, where the standard endpoints are growth and survival. At the end of a 7 day exposure, fish behavior was examined by feeding trials and behavioral quantification of movement using Noldus Ethovision. In the second study, sub-adult FHM and juvenile bluegill (Lepomis macrochirus) were exposed to five nominal treatment levels of DPHM for 1, 2, 4, 7 or 14 days and then behavioral activity and histamine response were quantified. Results from this study highlight the importance of employing mammalian pharmacokinetic/dynamic information for predicting comparative DPHM response thresholds in fish models, which may differ in conservation of pharmacological targets.

PS 2401 BEHAVIORAL, REPRODUCTIVE, AND GENOMIC RESPONSES TO NEUROPHARMACEUTICALS AT SUBCLINICAL CONCENTRATIONS FOUND IN THE ENVIRONMENT: FISH AS INDICATORS OF POTENTIAL HUMAN CONSEQUENCES OF LOW-DOSE EXPOSURES.

R. Klaper. *School of Freshwater Sciences, University of Wisconsin Milwaukee, Milwaukee, WI.* Sponsor: R. Hutz.

Pharmaceuticals and personal care products (PPCP) have been found in surface waters globally, and have now been found in drinking water, popular fish species that are regularly consumed by humans. Although not acutely toxic at these environmental levels the sublethal impacts from chronic exposures are not well understood. Fluoxetine, a highly prescribed selective serotonin reuptake inhibitor, is a commonly found PPCP in environmental samples. We tested the effects of environmentally relevant concentrations on the reproduction, behavior, and gene expression in the brain of the toxicological model species Pimephales promelas (fathead minnow) during a 4 week chronic exposure. P. promelas, is the current model species for environmental toxicology and can provide information on potential impacts on humans due to the conservation of biochemical pathways. Our data demonstrate that the impacts of fluoxetine at low-level exposures is both dose and time dependent. Fluoxetine influences aggression, response to key stimuli, such as predators, food and mates, which significantly decreases with exposure; and obsessive behavior patterns, exemplified by next cleaning, quantifiably increase. Fluoxetine concentrations as low as 1 μg/l were found to significantly impact the behavior of the fathead minnow. The response in fish is similar to that of mammals, where early interference with serotonin levels increases depression and anxiety. Some have attributed these changes to merely serotonin regulation and potential endocrine responses, however we demonstrate in this study that genomic pathways associated with cell adhesion, neurogenesis, and plasticity in the brain are impacted by SSRI's at these levels and hormones are not. This is one of the first studies to examine how these pathways may be impacted by low-level exposures and the duration of exposure.

PS 2402 TOXICOLOGICAL MECHANISMS OF AZASPIRACID: AN EMERGING ALGAL TOXIN IN U.S. WATERS.

M. Twiner[1], P. Hess[2], R. El-Ladki[1], S. Butler[1] and G. Doucette[3]. [1]*Natural Sciences, University of Michigan, Dearborm, MI,* [2]*Département Environnement, Microbiologie & Phycotoxines, IFREMER, Nantes, France and* [3]*Marine Biotoxins Program, NOAA/National Ocean Service, Charleston, SC.*

Azaspiracids (AZA) are an algal toxin group known to accumulate in shellfish and represent an emerging human health risk. Although monitored and regulated in many European and Asian countries, there are no regulatory requirements or standards in many of the other countries where AZAs have recently been identified. In Puget Sound (WA, USA), the presence of AZAs in shellfish and in samples collected using solid phase adsorption toxin tracking (SPATT) disks provide compelling evidence of this emerging risk. Efforts are now underway to better define the effects and mechanism of action for the various AZA analogues. Our investigations have employed *in vitro* cell models to characterize the potential toxicological impacts of AZA1, AZA2 and AZA3, and the use of DNA microarrays (i.e., gene chips) has yielded valuable insights as to possible biochemical pathways targeted by AZA1. Studies with several mammalian cell types yielded low nanomolar EC_{50} cytotoxicity values, making the AZAs one of the most cytotoxic algal toxin groups known. Our current focus is on further defining the specific mechanism of action, which appears to involve inhibition of cholesterol biosynthesis. Future experiments will continue to characterize analogue-specific cellular and biochemical responses as well as the toxicological effects of AZAs on various model organisms such as bacteria, developing brine shrimp, and fish.

PS 2403 SEASONAL DYNAMICS OF COLORED DISSOLVED ORGANIC MATTER IN THE LENA, MACKENZIE, AND YENESEI RIVERS.

K. E. Goodman[2], S. A. Walker[1] and R. W. Amon[1]. [1]*Marine Science, Texas A&M University, Galveston, TX and* [2]*Natural Sciences, Albany State University, Albany, GA.* Sponsor: L. Wrensford.

In this study, colored dissolved organic matter (CDOM) was evaluated in three of the largest Arctic rivers including: the Lena, Mackenzie, and Yenesei to determine if we can distinguish between the different rivers and fingerprint them in the Arctic Ocean based on their CDOM signal. Excitation Emission Matrix (EEM) spectroscopy coupled to Parallel Factor Analysis (PARAFAC), a multi-linear regression technique was used to identify the different terrestrial and protein components within each river over the time frame of 2004-2006. We hypothesize that the highest amount of CDOM within these rivers will occur for the month of June (during spring freshet) and the lowest amount of CDOM will occur for the other two months measured (March and August). To test this hypothesis, we analyzed samples for these three months over the time scale mentioned above to observe the seasonal variability of the rivers. Data compiled from these rivers were later composed into a preliminary PARAFAC model in which a prominent terrestrial and protein component were identified as c1 and c5. Results show that for all samples studied, the terrestrial component had a higher intensity than the protein component. Within each component, it was shown that the Lena River had the highest intensity amongst the rivers shown, meaning that it had the highest CDOM concentration as well. For each river shown, the change in its CDOM intensity was apparent before and after the peak season with highest intensities being in June and the lowest occurring in March and August. These results suggest that seasonal variability in the rivers is indeed related to the spring freshet during which more organic matter is discharged from those rivers. Component 1 (c1) identified in the PARAFAC model was also identified in coastal waters of the Canadian Basin indicating that terrestrial components derived from river data can be used as tracers in the Arctic Ocean.

PS 2404 EFFECTS OF PCB CONTAMINATION ON ACTIVITIES OF OXIDATIVE STRESS ENZYMES IN *S. ATROMACULATUS* LIVERS.

K. Shortt[1], D. Sparks[2], D. Millsap[2] and J. B. Watkins[1]. [1]*Indiana University, Bloomington, IN and* [2]*U.S. Fish and Wildlife, Bloomington, IN.*

Water source contamination with polychlorinated biphenyls (PCBs) commonly affects basic cellular functions of exposed organisms. The purpose of this study was to determine whether PCB contamination causes oxidative stress in the liver of central Indiana native creek chubs (*Semolitus atromaculatus*). The impact of PCB contamination was measured by examining the activity of indicators of oxidative stress in *S. atromaculatus* livers. Sampled streams were sorted into reference (0.0-0.1 ppm fish tissue), low PCB (0.1-0.5 ppm fish tissue), medium PCB (0.6-1.0 ppm) and high PCB (>1.0 ppm) comparison groups. Additional fish were collected from one high level PCB site after remediation. The fish were collected using standard electroshock techniques during the mating season. The livers were removed, frozen with liquid nitrogen, and prepared as 5% cytosols for analysis. The activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase were determined spectrophotometrically. PCB contamination was found to have significant adverse effects on the hepatic activities of glutathione reductase and peroxidase of the fish from the medium concentration streams. Activities of catalase and glutathione reductase were significantly different in the high PCB concentration streams. The activity of glutathione peroxidase in the high concentration group approached significance (p= 0.078). Hepatic enzyme activities of creek chubs from remediated sites were similar to those from reference sites. Further analysis of the effects of PCBs on lifespan and health of *S. atromaculatus* is needed.

PS 2405 THE TOXIC EFFECTS OF CHROMIUM COMPOUNDS IN NORTH ATLANTIC RIGHT WHALE AND SPERM WHALE CELLS.

T. Li Chen[1, 2, 3], J. Martino[1, 2, 3], S. S. Wise[1, 2, 3], C. LaCerte[1, 2, 3], F. Shaffiey[1, 2], H. Xie[1, 2, 3], A. L. Holmes[1, 2, 3], K. McPhearson[1, 2], I. Kerr[4], R. Payne[4], S. D. Kraus[5] and J. P. Wise[1, 2, 3]. [1]*Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME,* [2]*Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME,* [3]*Department of Applied Medical Science, University of Southern Maine, Portland, ME,* [4]*Ocean Alliance, Lincoln, MA and* [5]*Edgerton Research Laboratory, New England Aquarium, Boston, MA.*

Analysis of heavy metals in skin biopsies collected from North Atlantic right whales and sperm whales shows that both species are exposed to relatively high levels of chromium (Cr), 7.1 ± 0.8 and 8.8 ± 0.9 ppm, respectively. We tested the toxic effects of the most biologically relevant Cr valence states, trivalent and hexavalent chromium compounds [Cr(III) and Cr(VI), respectively] on whale skin cells grown in culture. Cr(VI) compounds are well known human carcinogens and skin irritants, whereas Cr(III) compounds are believed to be non-toxic to humans. Our results show that hexavalent compounds cause both cell death and DNA damage in whale cells at levels that are comparable to the concentrations that affect human cells. They also show that at the levels comparable to those we found in whales, Cr(III) induces cell death. Our study suggests that both Cr(III) and Cr(VI) may be a health concern for the whales. We are currently investigating the potential genotoxic effects of the trivalent Cr compounds. This work was supported by Grant number NA03NMF4720478 from the United States Department of Commerce, NOAA (J.P.W.), NIEHS grant ES10838 (J.P.W.) and the Maine Center for Toxicology and Environmental Health.

PS 2406 TOXICITY AND BIOACCUMULATION OF MERCURY (II) CHLORIDE IN TWO GENERA OF EARTHWORMS.

A. C. Nichols and D. A. Steffy. *Physical and Earth Sciences, Jacksonville State University, Jacksonville, AL.*

In continuing research to investigate the use of earthworms to moniter mercury (Hg) movement into terrestrial food chains, earthworms from two different genera, Lumbricus terretris and Eisenia fetida, were grown in soils with differing concentrations of mercury (II) ions. Mercury was added to the soils as aqueous solutions of mercury (II) chloride. Soil Hg concentrations used with Eisenia earthworms were soil background, 15 mg of added mercuric ions per kg of soil, 35 mg/kg of soil and 50 mg/kg of soil. Soil concentrations used with Lumbricus were background, 25 mg of mercuric ions per kg of soil, 50 mg/kg, 100 mg/kg and 200 mg/kg. After two weeks of Hg exposure, no live Eisenia were found in the 35 mg/kg and 50 mg/kg treatment boxes. Worms of this genus grown in the 15 mg/kg treatment box had a Hg tissue concentration of 18.73 +/- 0.57 (mean +/- SD)micrograms of Hg per gram of freeze-dried tissue. After two weeks of Hg exposure, live Lumbricus worms were collected from all of the treatment groups. Mercury tissue levels in these worms (in micrograms of Hg per g of freeze-dried tissue) were 10.65 +/- 0.30 for the 25 mg/kg exposure group, 44.70 +/- 4.14 for the 50 mg/kg exposure group, 53.88 +/- 1.71 for the 100 mg/kg exposure group, and 272.29 +/- 33.68 for the 200 mg/kg exposure group. Worms of the genus Lumbricus were able to tolerate greater body burdens of Hg than those of the genus Eisenia. Mercury analysis was conducted using cold vapor atomic absorption spectrometry.

PS 2407 COMPARISON OF CHROMIUM TOXICITY ON HUMAN, STELLER SEA LION, SPERM WHALE, AND NORTHERN RIGHT WHALE SKIN CELLS.

C. LaCerte[1, 2, 3], T. Li Chen[1, 2, 3], A. Holmes[1, 2, 3], S. Wise[1, 2, 3], S. Kraus[4], F. Gulland[5] and J. P. Wise[1, 2, 3]. *[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME, [2]Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME, [3]Department of Applied Medical Science, University of Southern Maine, Portland, ME, [4]Edgerton Research Laboratory, New England Aquarium, Boston, MA and [5]The Marine Mammal Center, Sausalito, CA.*

Marine pollution poses a significant ecological and health problem. Chromium is a common marine pollutant with sources from leather tanning and textile dyeing industries. We present work done on two Cr(VI) compounds sodium chromate (soluble) and lead chromate (particulate) using primary skin cells from human, Stellar sea lion (SSL), Sperm Whale (SPW) and North Atlantic right whale (NARW). Our results show that when based on intracellular Cr concentration human cells are the most sensitive to soluble Cr(VI) induced cytotoxicity. At 500 uM, human cells show 50% relative survival while SSL, SPW and NARW cells showed 62, 75 and 75%, respectively. When considering genotoxicity, SSL cells are more sensitive to soluble Cr(VI). At 100 uM, Cr damaged 18 SSL chromosomes in 100 metaphases analyzed, while human, SPW and NARW cells showed only 10, 2 and 3 damaged chromosomes. When considering particulate chromium, NARW cells are most sensitive with respect to cytotoxicity and SSL cells are most sensitive with respect to genotoxicity. At 200 uM Cr, NARW cells showed 58% survival while human, SSL and SPW cells showed 81, 79 and 89% relative survival. At 100 uM, Cr damaged 13 chromosomes in 100 metaphases in SSL cells; while human, SPW and NARW cells showed only 7, 6 and 5 damaged chromosomes. As a human carcinogen our study suggest that Cr(VI) is also a health concern for marine mammals. This work was supported by NOAA Grants NA03NMF4720478 (J.P.W.) and NA03NMF4390041 (J.P.W.), NIEHS grant ES10838 (J.P.W.) and the Maine Center for Toxicology and Environmental Health.

PS 2408 CYTOTOXIC AND MUTAGENIC EFFECTS OF THE HAIR DYE REACTIVE RED 51 AND ITS DEGRADATION USING PHOTOELECTROCATALYSIS.

T. B. Zanoni[1], D. P. Oliveira[1], L. E. Fraga[2], M. B. Zanoni[2] and N. R. Pissuti[1]. *[1]Universidade de São Paulo, Ribeirão Preto, Brazil and [2]Quimica Analítica, UNESP, Araraquara, São Paulo, Brazil.*

In recent years there has been a major concern over the extensive use of synthetic colorants including hair dyes. Prediction of harmful effects of these compounds are very important, considering that human is permeable to these these chemicals they could be absorbed through dermis, reaching blood vessel.Although epidemiological studies suggest a relation between occupational exposure to hair dyes and incidence of cancer, little is known about the toxicity of these dyes to humans and to the environment due to the patent protection.In this work, we evaluated the cytotoxic and mutagenic effect of the hair dye Basic Red 51 (BR51), using trypan blue in HepG2 (hepatoblastome) cells and Salmonella assay. We also investigated the efficiency of photoelectrocatalysis as a technique for removing the dye and its toxicity.For the toxicity assessment of BR51 a dose response curve for 24 and 48 hours was calculated for HepG2 cells after incubation at 37 oC and 5% of Co2.Salmonella assay was performed using pre-incubation protocol using strain TA100.The photoelectrocatalytical degradation of a BR51 solution at 1.0x10-5 mol L-1 was performed using a W/WO3/TiO2 under visible light irradiation.The color removal was determined using a spectrophotometer UV-vis and the degradation of organic matter was monitored by determination of total organic carbon (TOC).Our results indicated that the dose of 0.5 mg/mL of BR51 induced 99% of cell death after 24 hours of exposure while after 48 hours BR51 at 0.25 mg/mL was enough to cause 90% of cell death.We also observed that BR51 has mutagenic potential for TA100. The photoelectrocatalysis treatment of BR51for 60 min was able to remove 100% of the color and after 120 min, 69.02% of TOC was removed. The results indicate that RB51should be use with caution and that the photoelectrocatalysis is a promising tool for the treatment of aqueous samples contaminated with this hair dye.

PS 2409 COMPARISON BETWEEN THE EFFICIENCY OF TREATMENTS BY PHOTOELECTROCATALYSIS AND CONVENTIONAL CHLORINATION UNDER AQUATIC TOXICITY ENDPOINT.

E. A. Ferraz[1], T. M. Lizier[2], M. B. Zanoni[2] and D. P. Oliveira[1]. *[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil and [2]Universidade Estadual Paulista Júlio de Mesquita Filho - UNESP, Araraquara, São Paulo, Brazil.*

Introduction: Azo dyes constitute the largest group of colorants used in industry, and is currently considered a matter regarding the environmental and public health. When launched in industrial effluents they could contaminate the environment, since they are not degraded by conventional treatment processes. Objectives: Compare the use of the photoelectrocatalysis with conventional chlorination as an alternative method for the treatment of aqueous samples that contain mutagenic azo dyes and evaluate their ecotoxic effects, using Disperse Red 1, Disperse Red 13 and Disperse Orange 1 as model. Methods: The dyes solutions were treated using two techniques: The photoelectrocatalytic oxidation was performed in a photoelectrochemical reactor equipped with water refrigeration using an ultra-thermostatic bath and Ti/TiO2 thin film electrodes. Conventional chlorination was performed with chlorine gas, simulating the drinking water production, according to the Brazilian law. We evaluated the aquatic toxicity of the original and treated dyes using Daphnia similis assay. The experiment was performed with 6 different doses (0.001 to 10 mg/L) in 4 replicates. For each replicate five young organisms (6 – 24 h old) were exposed to the dyes for 48 h. After this period immobilized Daphnia were counted. Results: The original dyes Disperse Red 1 and Disperse Red 13 had EC50 values increased after treatments, going from 1.12mg/L before treatment of the Disperse Red 1 to 5.34 mg/L after chlorination and 2.82mg/L after photoelectrocatalysis. To the dye Disperse Red 13, EC50 values varied from 0.78mg/L before treatment to 2.24mg/L after chlorination and 3.98mg/L after photoelectrocatalysis. Disperse Orange 1 was not toxic to D. similis. Conclusion: Both chlorination and photoelectrocatalysis should be used with caution for the treatment of effluents that will be discharged into aquatic systems.

PS 2410 MULTIPLE ANALYSES OF MEAT CONTENTS IN CANNED DOG FOOD AND VEGAN BONE TREATS.

M. Hsieh[2], C. Chou[1], P. Shih[1] and H. Hsieh[2]. *[1]Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan and [2]Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taichung, Taiwan.*

In human public health, consumers demand quality meat products that are honestly-labeled to assure meat safety, fair pricing and to comply with religious regulations. Such issues have been much less investigated for pet food products despite fraudulent adulteration and substitution of meat species have been a suspicion. In this study, 11 brands of internationally commercialized canine canned foods were initially screened by ELISA and confirmed with nested-PCR and PCR coupled with capillary gel electrophoresis-laser induced fluorescence (CGE-LIF) detection. The canned foods were either labeled as cattle or chicken flavored while in fact their contents contained two or more meat species including cattle, chicken, swine and sheep. In addition, 6 artificial vegan bone treats manufactured in the USA, Canada and Singapore were examined for the presence of any protein of animal origin. The ELISA results showed that only 1 out of the 11 tested samples completely matched the exact meat species stated on the label while the remaining samples contained 1 to 3 unmentioned species. In contrast, PCR results indicated that 5 of the 11 samples matched the exact label. Overall, 5 brands contained 4 meat species while only 1 brand claimed so, mostly failing to indicate pig, followed by ovine species. On the

other hand, 1 vegan bone treat was found to contain protein of avian origin supposedly from its natural chicken flavor. Analytically, PCR results were not always in consistent with ELISA results and CGE was shown to have improved resolution than the slab gel electrophoresis revealing more meat species in the same sample. In conclusion, there is a significant discrepancy between the labeled meat species and their actual contents in canned food products. Manufactures are advised to be more accurate in stating their product labels to avoid infringement with governmental regulation.

PS 2411 ELUCIDATING THE MECHANISM OF VITAMIN A UPTAKE IN TUMOR-PRONE, VITAMIN A-SENSITIVE TISSUES.

C. E. Schulte[1, 2] and M. J. Rowling[1]. [1]*Food Science and Human Nutrition, Iowa State University, Ames, IA and* [2]*Interdepartmental Graduate Program in Toxicology, Iowa State University, Ames, IA.* Sponsor: S. Hendrich.

Vitamin A (retinol) is essential for maintaining normal eye, brain, placenta, and skin function, and its active derivatives all-trans- (ATRA) and 9-cis-retinoic acid have powerful effects on cell proliferation and differentiation. The long-term goal of the present study is to understand how carcinogenesis initiated by food borne chemical carcinogen exposure can be reduced through the optimization of vitamin A trafficking and signaling in tissues prone to tumor development. Retinol, the major circulating retinoid, travels to peripheral tissues in complex with retinol binding protein (RBP). Recently, a retinoic acid responsive protein with no previous known biological function, STRA6, has been identified as an essential transmembrane receptor that mediates retinol uptake in retinal epithelial cells. The goal of the current study is to characterize the mechanism by which RBP-bound retinol is internalized by mammary, prostate, and colon epithelial cells, retinoid-sensitive tissues that are prone to tumorigenesis. We utilized siRNA to determine whether knockdown of STRA6 inhibits retinol-RBP uptake as assessed by tracer assays and fluorescent microscopy, as well as to assess the requirement of STRA6 for retinol-mediated differentiation, apoptosis, and cell cycle arrest. Our results have shown that treating cancer cells with all-trans-retinoic acid (ATRA) induced STRA6 and up-regulated retinol uptake from RBP. Additionally, we determined whether ATRA dose-dependently altered expression of CD1, CD3, CDK2 and CDK4, proteins that interact to regulate proliferation, apoptosis, and cell differentiation. Collectively these studies will offer new insight into the role vitamin A trafficking may have in the anti-proliferative actions of vitamin A derivatives.

PS 2412 VITAMIN D STATUS MAY BE COMPROMISED BY THE CONSUMPTION OF FOODS RICH IN PCBS AND DIOXINS: RESULTS FROM A POPULATION WITH A WIDE RANGE OF SEAFOOD INTAKE—THE NORWEGIAN FISH AND GAME STUDY.

M. A. Haugen, B. Birgisdottir, A. Brantsæter, H. K. Knutsen, H. E. Kvalem, J. Alexander and H. M. Meltzer. *Department of Food Safety and Nutrition, Norwegian Institute of Public Health, Oslo, Norway.* Sponsor: M. Løvik.

Populations living far to the north are highly dependent on food sources of vitamin D during winter months when little or no pre-vitamin D synthesis from sunlight takes place in the skin. Fish liver, seagull eggs and oily fish have historically been major contributors to dietary vitamin D for the coastal population of Norway; however, they have also exceptionally high concentrations of PCBs. The aim of this study was to investigate whether intake of vitamin D and subsequent levels of 25-hydroxyvitamin D in serum might be compromised by the concomitant intake of dioxin-like compounds (dl-compounds). Participants from 27 municipalities in Norway were invited on the basis of their intakes of foods potentially high in different contaminants (n=111). Other participants were randomly chosen (n=73). The subjects answered a validated food frequency questionnaire and donated blood samples. Vitamin D intake was strongly associated with serum 25(OH)D among participants who did not consume fish liver and/or seagull eggs (n=101) [β=1.06 (0.48,1.63)], but not among participants who consumed fish liver and/or seagull eggs (n=80) [β=0.52 (-0.05,1.08)]. The association strengthened [β=0.66 (0.12,1.21)] when adjusted for retinol from food, indicating interference in absorption or metabolism of vitamin D. In the group that did not consume fish liver and/or seagull eggs, the total intake of dl-compounds was low (median intake 5.5 pg/kg bw/week), despite moderate to high intakes of oily fish. To secure optimal vitamin D status while keeping the intake of dl-compounds low, a healthy diet should include the intake of supplemental vitamin D and a moderate intake of oily fish. This would also ensure the intake of a variety of other important nutrients supplied by seafood. Our results support present-day dietary advice that intake of highly contaminated food items should be restricted despite high nutrient content.

PS 2413 ULTRAVIOLET LIGHT (254 NM) INACTIVATION OF FOODBORNE PATHOGENS ON FOODS AND STAINLESS STEEL SURFACES.

D. M. Barton[1], P. Landrio[1], D. E. Fulford[1], W. J. Mackay[1], J. E. Sites[2], M. Musgrove[2] and C. H. Sommers[2]. [1]*Biology & Health Services, Edinboro University of Pennsylvania, Edinboro, PA and* [2]*Food Safety Intervention Technologies Research Unit, U.S. Department of Agriculture, Wyndmoor, PA.*

Ultraviolet Light (254 nm) is a U.S. Food and Drug Administration approved non-thermal intervention technology that can be used for decontamination of food surfaces. In this study the use of Ultraviolet Light (UV) at doses of 0.5 to 4.0 J/cm^2 to inactivate Salmonella *spp.*, *Listeria monocytogenes*, and *Staphylococcus aureus* surface-inoculated (10^2-10^5 CFU/g) on the surfaces of frankfurters, bratwurst, shell eggs, chicken drumsticks, boneless skinless chicken breasts, boneless pork chops, tomatoes, and Jalapeno peppers was investigated. The pathogens displayed similar UV sensitivities to foodborne pathogens on individual food products. Pathogen reductions ranged from approximately 0.5 log CFU/g on raw meat and poultry to almost 4 log CFU/g on tomatoes, while the pathogens were not recovered from stainless steel at a UVC dose of 0.4 J/cm^2. Use of UVC light should be given serious consideration as a technology for routine surface decontamination of food contact surfaces and appropriate food products.

PS 2414 CHARACTERIZATION OF SHIGA-LIKE PROTEIN TOXINS I AND II FROM *ESCHERICHIA COLI* O157:H7.

K. Nguyen[1], W. H. Tolleson[1], A. Mugengana[2] and W. B. Melchior[1]. [1]*Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR and* [2]*Department of Chemistry, Philander Smith College, Little Rock, AR.*

Shiga-like protein toxins (Stx-1 and Stx-2) are produced by the enterohemorrhagic *E. coli* (EHEC) strains associated with lethal infections in humans, such as *E. coli* O157:H7. EHEC infections can cause endemic diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome. To differentiate between dangerous infections caused by *E. coli* serotypes other than O157:H7, the CDC recommends detection of Shiga toxins in addition to culturing the organism on selective and differential media in all cases of acute community-acquired diarrhea. Stx-1 and Stx-2 are ribosome-inactivating protein toxins with AB_5 subunit stoichiometry. Their B subunits facilitate toxin uptake by target cells and their A subunits provide an *N*-glycosidase activity that catalyzes the hydrolysis of the *N*-glycosidic bond of a conserved adenine residue within the EF-2 binding domain of 28S rRNA, thereby inhibiting protein synthesis and promoting cell death. The *N*-glycosidase activity of Stx-1 and Stx-2 can be detected using mouse liver total RNA as a substrate, either by quantifying adenine release using HPLC or by using a combination of reverse transcription and quantitative real-time PCR to detect the abasic site in 28S rRNA. Adenine release assays showed a similar pH dependent profile for both Stx-1 and Stx-2, which exhibited optimal activity at pH 6.5. The influence of temperature on toxin-catalyzed adenine release was also tested from 25-95°C, and we observed optimal *N*-glycosidase activity for both toxins at 45°C. Both toxins were inactivated with no adenine release detected when incubated with 28S rRNA at 95°C. Adenine release was also increased 3-4 fold in reactions containing 20 mM $MgCl_2$, consistent with a substrate coordination role by Mg^{2+} ions in *N*-glycosidase catalysis. Our results provide fundamental parameters to optimize enzyme activity and increase the sensitivity of assay methods used for the detection of Stx-1 and Stx-2.

PS 2415 ADULTERATION OF COMMERCIAL FRUIT JUICES DETECTED BY HPAEC-CD.

M. Wilson[1] and W. R. LaCourse[2]. [1]*Toxicology, University of Maryland Baltimore, Baltimore, MD and* [2]*Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, MD.*

As the popularity of Amazonian fruit juices continues to increase due to their high poly-phenol and anti-oxidant concentrations, food safety and proper labeling remain a priority to fight against artificial adulteration of these expensive juices. Adulteration can occur in a variety of ways, but is often done by diluting the expensive juice with a cheaper, more readily available juice. This minimizes the effect on taste, while still reducing the cost for the manufacturer, but also alters its nutritional value. Açai (pronounced a-sigh-ee), a berry native to regions around the Amazon River, has a unique organic acid profile, which helps to impart the tartness of flavor and stability of the juice. An established anion exchange chromatography method using suppressed conductivity detection has been modified to characterize the individual organic acids and anionic components of not only açai juice but a variety of juices. Some organic acid components of Açai have been determined such as lactic, quinic, malic, tartaric, citric and isoctric acids while others remain elusive. Chloride and sulfate ions are also important detected components of Açai juice.

Isocitric acid is the acid in the highest concentration compared to other identifying components, including chloride. The açai juice profile differs from apple juice with a decreased concentration of malic acid but an increase in tartaric acid. Unique identifying peaks in each juice profile, as well as changes in the ratios of these organic acids and anionic components, can be used as evidence of adulteration of açai juice by other juices.

PS 2416 EVALUATION OF ARISTOLOCHIC ACID DERIVATES IN MILK BY LIQUID CHORMATOGRAPHY TANEM MASS SPECTROMETRY.

C. Juan[1, 2], A. Rittieni[1], A. Juan-Garcia[3], G. Font[2] and J. Mañes[2]. *[1]Scienza degli Alimenti, Università di Napoli "Federico II", Portici, Italy, [2]Medicina Preventiva i Salut Publica, Ciencies de l' Alimentació, Toxicologia I Medicina Legal, Valencia University, Burjassot, Valencia, Spain and [3]Basic Medical Science, Purdue University Cytometry Laboratories, West Lafayette, IN.*

Aristolochic acids (AAs) are a mixture of structural-related compounds (aristolochic acid I (AA I) and aristolochic acid II (AA II)) and correlated with Aristolochic acid nephropathy. AAs have pharmacological properties (anti-inflammatory, antitussive and antiplatelet aggregation) but herbal products containing AAs is banned in Europe because of the awareness of nephrotoxic,and carcinogenic to humans (Group 1). AAs in the animal diet may leave residues in edible animal tissues and milk, and consecuently undesirable effects on consumer health. We present a LC–MS/MS method was to determine AA I and AA II in bovine and caprine milk. A reversed phase column Gemini C18 with linear gradient program with water and methanol (0.2% acetic acid and 1 mM ammonium acetate) was used. AAs could be determined within 20 min. Under optimum LC–MS/MS conditions, the limit of detection was 3 and 8 ng/mL for AA I and AA II, respectively, and the limit of quantification 5 and 10 ng/mL for AAI and AA II, respectively. Run-to-run repeatability and intermediate precision of peak area for AA I and AA II were less than 5.74% relative standard deviation (RSD). Accuracy was tested by spiking 10, 100 and 1000 ngmL−1 in milk and the recoveries were within 76.5–92.9%. Matrix effects were within 78.8–127.7%. The developed method was successfully applied to determine AA I and AA II in several bovine and caprine milk samples. LC–MS/MS method developed is efficient, sensitive, and accurate for AAs determination in milk. The method appears to be suitable for safety assurance and quality control of commercially available samples. Acknowledgment: Conselleria de Educació of Generalitat Valenciana for the financial support of "Programa VALi+d per a investigadors en fase postdoctoral (APOSTD) 2010".

PS 2417 RETAINED BACTERICIDAL ACTIVITY OF DIPHENYLIODONIUM CHLORIDE IMPREGNATED IN ALGINATE BEADS FOLLOWING EXPOSURE TO STOMACH-SIMULATING PH CONDITIONS.

S. V. Epps[1], N. A. Krueger[2], T. D. Phillips[1], R. B. Harvey[2], J. Taylor[1], R. C. Anderson[2] and D. J. Nisbet[2]. *[1]Veterinary Integrative Biosciences/Toxicology, Texas A&M University, College Station, TX and [2]USDA/ARS, Southern Plains Agricultural Research Center, College Station, TX.*

Campylobacter are a leading cause of bacterial derived human foodborne illness. The deaminase inhibitor, diphenyliodonium chloride (DIC), markedly reduces survivability of *Campylobacter in vitro*, but results from *in vivo* studies with swine and cattle indicated that absorption of DIC within the stomach and small intestine precluded delivery of efficacious quantities of this compound to the primary sites of *Campylobacter* colonization within animals, the cecum and large intestine. Thus, in an attempt to develop a suitable product capable of bypassing absorption within the stomach and small intestine, we prepared and evaluated the bactericidal activity of alginate bead preparation impregnated with 0.05 mM DIC following exposure to an acidic solution (pH 3.8) simulating that of the stomach. The DIC-impregnated beads were prepared by mixing 2% (wt/vol) sodium alginate with 0.1 mM DIC in water and with combining this in a dropwise fasion with an equal volume of 1 M calcium chloride. Exposure to the low pH was accomplished by reacting 5 g of beads with 5 ml buffered pH solution for 2 h after which time the beads were removed and distributed, 1.0, 0.1 or 0.01 g, to tubes containing 9 ml Bolton broth and inoculated with approximately 1,000,000 colony forming units (CFU) of *C. jejuni*. After 24 h incubation at 42 degrees C, the incubations containing DIC-impregnated alginate beads retained 100, 76 and 98% bactericidal activity as that determined with positive control cultures incubated likewise except with 0.05, 0.005 and 0.0005 mM added DIC, which achieved mean ± SD decimal log reductions in *C. jejuni* concentrations of 6.72 ± 0.01, 5.83 ±1.26 and 0.17 ± 0.27, respectively. These results indicate that impregnation of 0.05 mM DIC in sodium alginate beads may be suitable to bypass the low pH environment of the stomach.

PS 2418 ACUTE AND SUB-CHRONIC TOXICITY OF USNIC ACID IN MICE AND RATS.

J. E. Seng[1], A. Babb[1], A. Ali[1], S. M. Lewis[2], H. Badgley[1], G. Olson[1] and J. E. Leakey[2]. *[1]Toxicologic Pathology Associates, Jefferson, AR and [2]Office of Scientific Coordination, National Center for Toxicological Research, Jefferson, AR.*

Usnic acid (UA) is a secondary metabolite present in most species of Usnea lichens (UL). Since UA is a potent uncoupler of electron transport across biological membranes, UL have been used as natural bactericidal agents in traditional medicine. UA has also been used as a fat burning weight loss agent due to its ability to uncouple mitochondrial oxidative phosphorylation but this use of UA has resulted in severe hepatotoxicity in certain individuals. Pure UA and preparation of ground UL containing equivalent concentrations of UA were tested for acute and subchronic toxicity in B6C3F1 mice and Fischer 344 rats. After 14-days of continuous exposure to feed containing 180 ppm to 1250 ppm UA or UL, toxicokinetic analysis of hepatic and serum UA concentrations was performed. In a 90-day subchronic study of UA and UL, mice and rats were administered 15 ppm to 360 ppm or 30 ppm to 720 ppm in feed, respectively, and standard toxicological parameters were examined. Toxicokinetic analysis demonstrated that after 14-days of treatment UA accumulates to high concentrations in blood and liver. After 90-days of oral (feed) dosing, UA administration had no impact on mouse survival, whereas rats exposed to 720 ppm UL did not survive beyond the first half of the study. There were no histopathological subchronic effects of UA in mice, but dose-related hepatocellular degeneration was observed in UA-treated male rats as well as UL-treated mice and rats. Additional pathology was noted in UL-treated animals including the reproductive system as well as corresponding clinical observations. Clinical pathology associated with hepatotoxicity was not considered biologically significant. These studies demonstrate pure UA exhibits differential toxicity when compared to UL. [The work was supported by the IAG #224-07-0007 between the FDA/NCTR and the NIEHS/NTP].

PS 2419 NOVEL FEEDING BIOASSAY FOR CHARACTERIZATION OF DEOXYNIVALENOL-INDUCED FEED REFUSAL IN THE MOUSE.

B. Flannery[1, 2] and J. Pestka[1, 3]. *[1]Food Science and Human Nutrition, Michigan State Univeristy, East Lansing, MI, [2]Center for Integrative Toxicology, Michigan State University, East Lansing, MI and [3]Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.*

Deoxynivalenol (DON) is a trichothecene mycotoxin that is resistant to heat processing, and as a result, contaminates grain products worldwide. DON causes feed refusal and growth retardation, the latter of which has been used to establish a Tolerable Daily Intake (TDI) in Europe. Despite being a primary adverse effect, the mechanisms of DON-induced feed refusal are not well understood. In order to relate feed refusal to other DON-induced physiological changes, the dose and timing at which feed refusal occurs needs to be elucidated. Using a novel mouse feeding bioassay, we determined DON's short-term effects on feed refusal following intraperitoneal injection. DON caused a significant, dose-dependent reduction in food intake that could be measured with-in two hours of exposure. In naïve mice, the lowest dose that caused feed refusal was 0.5 mg/kg bw. At 1 mg/kg bw DON, the duration of feed refusal was 3 hr; while at 5 mg/kg bw feed refusal lasted 6 hr. Mice appeared to become resistant to DON-induced feed refusal upon subsequent 24 hr exposures; yet, mice re- exposed to DON (5 mg/kg bw) 14 days after the last exposure exhibited a feed refusal response comparable to that of naïve mice. Taken together, these data suggest transient DON-induced feed refusal. Furthermore, these data suggest that feed refusal and possibly other DON-induced physiological changes, can be affected by the number of DON exposures and time since the last exposure. This information is important because risk assessments of DON have been based on chronic feeding studies.

PS 2420 COMPARISON OF THE DOSE-RESPONSES OF 7- AND 28-DAY CO-EXPOSURES TO MELAMINE AND CYANURIC ACID IN F344 RATS.

G. Gamboa da Costa[1], C. C. Jacob[1], L. VonTungeln[1], G. Olson[4], D. G. Hattan[3], R. Reimschuessel[2] and F. A. Beland[1]. *[1]U.S. FDA National Center for Toxicological Research, Jefferson, AR, [2]U.S. FDA Center for Veterinary Medicine, Laurel, MD, [3]U.S. FDA Center for Food Safety and Applied Nutrition, College Park, MD and [4]Toxicologic Pathology Associates, Jefferson, AR.*

The intentional adulteration of pet food ingredients with melamine (MEL) and a number of its derivatives, including cyanuric acid (CYA), caused kidney failure and death of hundreds of cats and dogs in the USA. Co-exposure to low levels of these compounds elicits nephrotoxicity due to the formation of melamine cyanurate

crystals in the nephrons. In previous experiments we evaluated the dose-response for dietary co-exposure to MEL and CYA for 7-days in F344 rats. We report here the dose-response obtained after a period of 28-days in F344 rats using comparable experimental conditions. Rats were provided feed fortified with 0 (control), 30, 60, 120, 180, 239, or 359 ppm of melamine and cyanuric acid, comparable to approximately 2.5, 5, 10, 15, 20 and 33 mg/kg bw. As assessed by histopathology, the lowest dose that produced alterations was 120 ppm, versus 229 ppm in the 7-day study. Wet-mount analysis of kidney sections, however, demonstrated the formation of melamine cyanurate spherulites in 1 of 24 rats at the 30 ppm dose and in 3 of 23 rats at the 60 ppm dose. Clinical chemistry and body weight were less sensitive endpoints. While the determination of a specific NOAEL or TDI from this study is a function of the endpoints considered, these data demonstrate that the length of exposure plays a role in the definition of a threshold of toxicity for these compounds, a fact that should be taken into consideration when conducting future risk assessments. Funded in part by NTP IAG 224-07-0007 between the FDA/NCTR and the NIEHS/NTP.

PS 2421 A 90-DAY DIETARY TOXICITY STUDY OF R, R-MONATIN SALT, A NATURAL HIGH POTENCY SWEETENER, IN RATS.

J. Hlywka[1], W. A. Brathwaite[1], A. I. Nikiforov[2], M. L. Rihner[2] and A. K. Eapen[3]. [1]*Cargill Inc., Wayzata, MN,* [2]*Toxicology Regulatory Services, Charlottesville, VA and* [3]*WIL Research Laboratories LLC, Ashland, OH.*

The root bark of Schlerochitin ilicifolius, a plant native to South Africa, contains an intensely sweet substance identified as R,R-monatin. While S. ilicifolius has been consumed by indigenous populations historically, the toxicity profile of R,R-monatin is generally unknown. To contribute data relevant to evaluation of the safety of this natural sweetener for use in food, the subchronic toxicity of sodium/potassium (2R, 4R)-2-amino-4-carboxy-4-hydroxy-5-(3-indolyl) pentanoate, otherwise R,R-monatin salt, was assessed in Sprague-Dawley rats. Male and female rats (20 animals/sex/dose) were exposed to concentrations of 0, 5,000, 10,000, 20,000 or 35,000 ppm (0, 0.5, 1.0, 2.0 or 3.5% w/w) R,R-monatin salt in the diet for 91 consecutive days. Functional observational battery and motor activity assessments revealed no test article-related alterations. There were no toxicologically relevant clinical, macroscopic, or histopathological findings in any of the test article-treated groups. However, mean body weights in the 35,000 ppm group males and females were 7% and 12% lower (statistically significant), respectively, than the control group at study week 13. In the absence of other observations associated with systemic toxicity and lower food consumption, the magnitude of the body weight difference in the 35,000 ppm group females relative to the control group exceeded 10%, which is generally considered to be an adverse finding. Therefore, based on the results of this study, the no observed adverse effect level (NOAEL) for oral (dietary) administration of R,R monatin salt to female rats for a minimum of 91 consecutive days was 20,000 ppm (equivalent to an exposure level of 1,544 mg/kg/day). In the absence of evidence of systemic toxicity following dietary administration of R,R monatin salt to male rats for a minimum of 91 consecutive days, the NOAEL was 35,000 ppm (equivalent to an exposure level of 2,368 mg/kg/day), the highest dietary concentration used in this study.

PS 2422 EVALUATION OF THE HYPERSENSITIVITY POTENTIAL OF ALTERNATIVE BUTTER FLAVORINGS: ARE THEY SAFE SUBSTITUTES FOR DIACETYL?

S. Anderson, J. Franko, E. Lukomska and B. J. Meade. *NIOSH, Morgantown, WV.*

Concern has been raised over the potential association of diacetyl, a chemical used in the production of butter flavorings, with lung disease clinically resembling broncholitis obliterans in food manufacturing workers. This has resulted in the need for identification of alternative chemicals to be used in the manufacturing process. We have previously shown diacetyl to be a dermal irritant and sensitizer (EC3 1.9) when tested in a combined murine local lymph node assay (LLNA). Structurally similar chemicals, 2,3-pentanedione 2,3-hexanedione 3,4-hexanedione and 2,3-heptanedione, used as constituents of synthetic flavoring agents have been suggested as potential alternatives for diacetyl. Immunotoxicity data on these chemicals are limited. The present study evaluated the dermal irritation and sensitization potential of diacetyl alternatives using a murine model. None of the chemicals were identified as dermal irritants when tested at concentrations up to 50%. Similar to diacetyl, concentration-dependent increases in lymphocyte proliferation were observed following exposure to all four chemicals, with calculated EC3 values of 15.4% (2,3-pentanedione), 18.2% (2,3-hexanedione), 15.5% (3,4-hexanedione) and 14.1% (2,3-heptanedione). No biologically significant elevations in total serum IgE were identified, however significant elevations in local IgE (IgE+B220+ lymphocytes), IL-4, and INF-gamma production by stimulated draining lymphocytes were observed after exposure to 25-50% concentrations of these chemicals. These results demonstrate the potential for development of hypersensitivity responses to these proposed alternatives and raise concern about their use as a replacement for diacetyl as flavoring agents.

PS 2423 BEAUVERICIN-INDUCED OXIDATIVE STRESS IN CHO-K1 CELLS IS PREVENTED BY VITAMINS AND FLAVONOIDS.

G. Font[1], L. Haro[1], J. Mañes[1], A. Anadón[2] and M. J. Ruiz[1]. [1]*Laboratorio de Toxicología, Universidad de Valencia, Burjassot, Valencia, Spain and* [2]*Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain.*

Mycotoxins affect cellular processes including inhibition of cell proliferation, oxidative stress and lipid peroxidation. The hypothesis that vitamin C, vitamin E, quercetin and pterostibene could prevent the mycotoxins-induced oxidative stress in the Chinese Hamster Ovary (CHO-K1) cells was investigated. The CHO-K1 cells exposed to the emergent beauvericin (BEA) mycotoxin (0.5 and 1.0μM for 24h) were simultaneously treated with vitamins C and E, quercetin and pterostilbene (2.5-50μM). MTT was used as indicator of cell viability, and determination of malonedialdehyde (MDA)– thiobarbituric acid (TBA) adduct was signed to determine lipid peroxidation. BEA cytotoxic effect was quantified as the concentration required for inhibiting cell growth by 50%(IC50). The IC50 value of BEA in CHO-K1 cells incubated for 24h was 12.08±1.10μM. Moreover, BEA increased significantly the production of MDA after 24h of treatment with 0.5 and 1μM (≈45%). However, when BEA and antioxidant are present simultaneously in cell culture, this will boost antioxidant activity. Vitamins were effective on decreasing MDA production at the lower concentration tested but not at the higher. On the other hand, pterostilbene shows protective effect only at lowest concentration tested, while quercetin showed it at all the concentrations. These results could be due to the ultimate balance between potentiating and protective effects may depend on the subtle redox equilibrium within the cells and the balance within the complete cellular antioxidant network. The results suggest that BEA exposure on CHO-K1 cells resulted in cytotoxicity and free-radical or other reactive species-mediated cell damage in a dose-dependent way. Oxidative stress was prevented by simultaneous treatment with vitamins and flavonoids. Quercetin was the most effective during BEA exposure. Supported by Conselleria de Sanitat (EVES2010-028) and Science and Innovation Spanish Ministry (AGL2010-17024/ALI)

PS 2424 SIMULTANEOUS DETERMINATION OF TYPE B TRICHOTHECENE MYCOTOXINS AND THEIR DERIVATIVES IN WHEAT BY LC/MS/MS.

T. Kadota[1], M. Kimura[2], S. Hirano[1], O. Tajima[1], T. Nakajima[3], Y. Kamata[4] and Y. Sugita-Konishi[4]. [1]*Kirin Holdings Co., Ltd., Kanagawa, Japan,* [2]*RIKEN ASI, Saitama, Japan,* [3]*National Agricultural Research Center for Kyushu-Okinawa Region, Kumamoto, Japan and* [4]*Division .Microbiology, NIHS Japan, Tokyo, Japan.*

Type B trichothecene mycotoxins are toxic metabolites produced by Fusarium fungi that contaminate agricultural crops. Trichothecenes are a large family of sesquiterpene mycotoxins and many derivatives are present in the biosynthetic pathway. There are concerns that these derivatives have toxicity and reside at high concentrations in agricultural products. In this study, we developed a LC/MS/MS method for the determination of type B trichothecene mycotoxins and their derivatives in wheat. Derivatives of type B trichothecene, isotrichodermin, calonectrin (CAL), 3-deacetyl-CAL, 15-deacetyl-CAL, 3,15-diacetyl-NIV, 4,15-diacetyl-NIV, 3,15-diacetyl-DON, and 3,4,15-triacetyl-NIV were purified from trichothecene blocked mutants. DON, NIV, 3-acetyl-DON, 15-acetyl-DON, fusarenon-X and DON-3-glucoside standards were prepared from the commercial products. Wheat samples were extracted with acetonitrile/water/acetic acid (85:14:1) and the extract was treated with multifunctional column. The identification and determination of each trichothecene were achieved by LC/MS/MS system. Chromatographic separation of each toxin was performed by using a C18 column with a linear gradient of methanol and water contained 10 mM ammonium acetate. Trichothecene mycotoxins were purified with multifunctional column cleanup procedure, and good recoveries ranging 84.3–110% and RSDs (0.4–7.2%) were obtained in recovery tests. The limit of detection and quantification in wheat were 0.03–1.4 and 0.1–4.7 ng/g, respectively. The contaminated wheat samples inoculated by fungi in the field were measured for the screening of trichothecenes and their derivatives. The results revealed that derivatives and metabolite (DON-3-glucoside) were detected along with DON and NIV. Accordingly, LC/MS/MS method developed in this study is a practical method for the determination of type B trichothecene mycotoxins and their derivatives.

PS 2425 EFFECTS OF FUMONISINS IN ETHANOL PRODUCTION.

E. Bilsten and G. Munkvold. *Plant Pathology, Iowa State University, Ames, IA.*

Fumonisins are common maize contaminants produced by a number of fungal species in the genus Fusarium; consumption of fumonisins leads to a variety of detrimental health effects in livestock. Dried distiller's grains and solubles (DDGS) are a co-product of ethanol production used for animal feed and revenue generated from their sale is essential to profitability in the ethanol industry. Studies conducted in 2007-2009 showed fumonisins were more concentrated in DDGS as compared with the original grain, though the degree of concentration varied. The Grain Inspection, Packers and Stockyards Administration (GIPSA) has approved quality testing methods for fumonisin concentrations in grain intended for animal feed, including testing by ELISA methods. Analysis of fumonisin contaminated grain (and consequent DDGS) was performed using both a GIPSA-approved ELISA test kit and HPLC-fluorescence detection of the naphthalene-2,3-dicarboxalehyde (NDA) derivatized fumonisin molecule. There was a close correlation between ELISA and HPLC results (R = 0.97) but ELISA tended to underestimate fumonisin concentrations. Under-estimation of fumonisins by ELISA testing could result in feeding animals more highly contaminated grain than is considered safe. Use of fumonisin-contaminated maize for ethanol production may result in decreased quality and marketability of co-products, and may also impact fermentation efficiency and subsequent ethanol yields.

PS 2426 COMBINATIVE TOXICITY OF MYCOTOXIN MIXTURES IN ANIMALS AND HUMAN CELLS.

G. Qian, L. Tang and J. Wang. *Environmental Health Science, University of Georgia, Athens, GA.*

Mycotoxins are naturally occurring toxic fungal metabolites, which have caused the tremendous economic loss worldwide and are etiological agents of a variety of animal and human toxicoses. The aflatoxins, certain tricothecenes, and fumonisins are ubiquitous food contaminants and have been shown to be carcinogenic and/or teratogenic in experimental animal models. Because of their potent toxicities in animal and humans, some mycotoxins are candidates for use as biological warfare agents, for example, aflatoxin and T-2 toxin have been known to be weaponized and may be available for use as terrorist attack(s); therefore, there is an urgent need to understand the combinative toxicity of these toxin mixture(s). In this study, combinative toxicities of aflatoxin B1 (AFB1), T-2 toxin (T-2), and fumonisin B1 (FB1) were tested in F-344 rats, mosquitofish (Gambusia affinis), and human hepatoma cells (HepG2) and bronchial epithelial cells (BEAS-2B). Preliminary experiments were conducted in order to assess the acute toxicity/cytotoxicity and obtain LD50, LC50 and IC50 values for individual toxins in each model, respectively. This was followed by testing combinations of mixtures containing AFB1, T-2, and FB1 to obtain LD50, LC50 and IC50 values for the combination in each model. All models demonstrated a significant dose response in observed parameters to treatment. The potency of the tri-toxin mixture was gauged through the determination of the combinative toxicity index (CTI=estimated LD50, LC50 and IC50/measured LD50, LC50 and IC50). Additive toxic effects were observed in rat for the tri-toxin mixture (CTI=1.0). Synergistic toxic effects were found in mosquitofish with the CTI of 2.8. Additive to synergistic toxic effects were observed in HepG2 cells (CTI=1.8) and BEAS-2B cells (CTI=2.0) for the tri-toxin mixture. These results will provide foundational knowledge in future studies on long-term combinative toxic effect and risk assessment of health effect of co-exposure to these mycotoxin mixtures. (Supported by the research contract, DAAD13-02-C-0070, from DOD).

PS 2427 MYCOTOXINS MULTI-CONTAMINATION OF PRESCHOOL AGE CHILDREN IN BENIN: PRELIMINARY STUDY IN THE ZOU REGION.

E. E. Creppy[1], J. Tabe-Dumond[1, 2], S. Moukha[1, 2] and B. Sangare-Tigori[1, 3]. *[1]Toxicology, University Bordeaux 2, Bordeaux, France, [2]Toxicology, University Bordeaux 2, Bordeaux, Gironde, France and [3]Toxicology Department, University of Abidjan, Abidjan, Côte d'Ivoire.*

The natural occurrence of mycotoxins in food and foodstuffs is widespread, and of human health concern, especially in tropical regions. In an attempt to confirm mycotoxins multi contamination, 25 samples of cornstarch and mixed foods intended for children consumption, (apparently healthy children, n=20) and (malnourished children n=49) from the Zou-Region nutrition recovery centres in Benin were assayed for (ochratoxin A, aflatoxins, zearalenone, T-2 toxin, HT-2 toxin, nivalenol, deoxynivalenol and fumonisins) by LC-MS-MS. All the samples were contaminated by several mycotoxins, with concentrations ranging from 0.5 – 2879.2 μg/kg for aflatoxins, 50 – 1290 μg/kg for trichothecenes, 0.5 – 1360 μg/kg for fumonisins, 0.5 – 4.3 μg/kg for ochratoxin A and 5 – 22 μg/kg for zearalenone. Ochratoxin A (OTA), known to be nephrotoxic, immunosuppressive and carcinogenic is a mycotoxin produced by fungi of Aspergillus and Penicillium genera. It has been assessed in blood by enzyme immunoassay after purification using immunoaffinity columns followed by confirmation by HPLC-Fluorimetric quantification and taken as a biomarker of exposure of children to all the above mentioned mycotoxins. When ingested, this toxin has a bioavailability of about 50%. All children tested are contaminated, 100% of well nourished ones show OTA blood concentrations from 6.9 to 54 μg/l, whereas, malnourished children showed significantly lower concentrations (p= 0.0051), in the range of 0.3 – 48.4 μg/l and a mean value of 20.8 μg/l. Ochratoxin A found in the children's blood fully reflect OTA contamination of their diet, and is a biomarker of other mycotoxins also found in foodstuffs. The present data confirm mycotoxins multi contamination in west-Africa and show for the first time that trichothecens and zearalenone are present in foodstuffs in Benin and may be worsening the adverse effects of aflatoxins and ochratoxins in children of preschool age in Benin.

PS 2428 PYRROCIDINE A TOXICITY IN MICE.

S. Hooser[1], C. R. Wilson[1], G. N. Burcham[1], W. M. Haschek-Hock[2] and D. T. Wicklow[3]. *[1]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN, [2]Department of Pathobiology, University of Illinois, Urbana, IL and [3]Bacterial Foodborne Pathogens and Mycology Research Unit, USDA ARS, Peoria, IL.*

Pyrrocidines A and B are metabolites produced by the fungus Acremonium zeae, a common seedborne endophyte of corn. Pyrrocidine A exhibits potent activity against Gram-positive bacteria, including drug resistant strains, and displays significant activity against Candida albicans, as well as major stalk and ear rot pathogens of corn. Pyrrocidines have been detected in visibly molded corn subjected to drought and temperature stress and are consistently produced in A. zeae isolates from corn grown in warmer regions. A. zeae is being evaluated for its potential application as a biocontrol agent in protecting corn plants from virulent pathogens. Thus, information is needed to determine whether or not increased pyrrocidines in corn would pose a safety risk. In 2007 the Haschek laboratory reported that the cytotoxicity of pyrrocidine A to human HepG2 cells was more potent than the known Fusarium mycotoxins, deoxynivalenol, fumonisin B1, moniliformin and zearalenone. However, pyrrocidine A toxicity was not observed in mice at doses up to 10 mg/kg body weight based on clinical signs, organ weights, and gross and microscopic lesions. The studies reported here evaluated higher doses in mice. Adult, male, Swiss-Webster mice were administered pyrrocidine A (n = 5/group) at 0 (DMSO control), 10, 100 or 250 mg/kg (i.p.). Pyrrocidine A at 10 mg/kg was not lethal and did not cause clinical signs, while 100 mg/kg and 250 mg/kg resulted in death within 24 hours. Pyrrocidine A at 25 mg/kg and at 50 mg/kg caused death in 1 mouse in each group within 12 hours. No significant gross or histological lesions were seen. This study indicates that at doses of 10 mg/kg (i.p.), pyrrocidine A does not cause acute toxicity but can be fatal at doses of 25 mg/kg or greater.

PS 2429 FOOD ANTIOXIDANTS HAVING NO, OR WELL-SPECIFIED, LEVELS OF ESTROGENIC ACTIVITY (EA).

C. Z. Yang[1] and G. Bittner[1, 2, 3]. *[1]CertiChem, Inc., Ausin, TX, [2]PlastiPure, Austin, TX and [3]University of Texas, Austin, TX.* Sponsor: R. Tice.

Food antioxidants (AOs) have for decades been added in various combinations (formulations) to foodstuffs primarily to preserve food quality and more recently for health benefits such as reducing the incidence of some cancers, brain damage, and cell ageing. Chemicals such as AOs having agonist or antagonist EA at concentrations used in foodstuffs (mM to μM) often have adverse effects on mammals, including humans. Fetal or juvenile mammals are especially sensitive to effects of chemicals having EA at very low dosages (nM to pM concentrations). Using a recently-developed sensitive and reliable robotic assay, we show that many, synthetic or natural, commonly-used food AOs have significant EA and that many other, synthetic or natural, less-commonly-used AOs do or do not have EA. For example, BHA and BHT have easily detectable agonist EA whereas propyl gallate has easily detectable antagonist EA. Using such data, CertiChem is devising food AO formulations having no detectable EA—or well-specified levels of EA. The creation of such formulations is important because some human populations such as pregnant mothers, infants and juveniles should almost-certainly not ingest foodstuffs containing AO formulations having easily-detectable levels of EA. Conversely, some conditions in adult humans (e.g., menopause, some cancers or abnormalities of the prostate) would probably be ameliorated by foodstuffs containing AO formulations having well-specified levels of EA. Supported by NSF SBIR Phase I 0912601 and NIH SBIR44ES014806.

PS 2430 TOXICITY OF RIBBON WORM *CEPHALOTHRIX* SPECIES ON THE SURFACE OF THE SHELL OF CULTURED OYSTERS IN HIROSHIMA BAY, HIROSHIMA PREFECTURE, JAPAN.

M. Asakawa. *Graduate Schoool of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan.*

Paralytic toxicity of a species of ribbon worms identified as a member of the genus *Cephalothrix*, found on the shells of cultured oysters in Hiroshima Bay, was examined between November and May from 1998 to 2005 by the standard bioassay method for tetrodotoxin (TTX). The toxicity study showed that all of the 764 specimens examined were found to contain toxins with strong paralytic action in mice throughout the season covered. Their toxicity scores ranged from 169 to 25,590 mouse units (MU) per gram for whole body. About eighty percent of the total individuals were ranked as 'strongly toxic' (≧1,000 MU/g). Forty-eight percent of the total specimens possessed toxicity scores more than 2,000 MU/g. The highest toxicity detected was 25,590 MU/g in a specimen collected on June 25, 1999. The total toxicity of this particular specimen was roughly calculated to be 5,631 MU, the value that is approximately equivalent to half of the minimum lethal dose of TTX in human. Some seasonal variations of toxicity of this ribbon worm could be recognized. Specimens collected on October 28, 2004 to January 20, 2005 showed mean toxicities of 1,855-4,076 MU/g. But on February 22, 2005, their toxicities had sharply decreased down to a mean of 1,578 MU/g. At the beginning of December, their toxicities increased to a mean of 4,510 MU/g. This paralytic toxin was isolated and recrystallized. The recrystallized toxin showed a specific toxicity of 3,520 MU/mg. This toxin was finally identified as TTX by the instrumental analysis. As the culturing of edible bivalves such as oysters and scallops is a flourishing industry in Japan, surveillance for the distribution of toxic ribbon worms in other areas besides Hiroshima Bay is urgently needed.

PS 2431 DIFFERENTIAL HEAT STABILITY OF RACTOPAMINE IN LIQUID AND PROTEIN MATRICES.

C. Chou[1], P. Lan[1], M. Hsieh[2] and W. Chang[3]. [1]*Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan,* [2]*Graduate Institute of Microbiology and Public Health, National Chung-Hsing University, Taichung, Taiwan and* [3]*Animal Disease Diagnostic Center, National Chung-Hsing University, Taichung, Taiwan.*

Ractopamine (RAC) is a β-adrenergic agonist approved to be used as a growth promoter in livestock production in the USA, Australia and Canada. However, RAC is banned in Europe, China and Taiwan due to its potential risks linked to adverse cardiovascular effects and deaths caused by residues in visceral tissues. In order to have a more accurate estimation of consumer exposure to RAC, its heat stability was evaluated in different heating processes (water bath, microwave, autoclave), heating lengths (100°C: 15, 30, 60, 120 min), and matrices (water, soybean sauce, beef, pork, and swine liver, kidney, lung). RAC degradation was determined by high performance liquid chromatography with UV detection. Results indicated that regardless of heating processes, RAC degradations in water were all below 13.1%, but structural degradation was accelerated in soybean sauce (35.5%). In protein matrices, degradation also increased and ranged from 24.4-46.4%. Heat stability was ranked as beef>beef/pork in soybean sauce>swine liver>swine kidney >pork>swine lung, indicating differential heat stability of RAC in different protein matrices, with significantly lower ($p<0.05$) degradation in beef than in pork. Although thermodegradation of RAC was significantly higher in lung than in liver and kidney tissues, the markedly higher (several hundred folds) residue level of RAC reported in lung still rendered it the most risky tissue for RAC exposure. The protein-binding of RAC to cattle (10.5%) and swine (7.4%) plasma were low as determined by microdialysis, suggesting that RAC degradation might not be protected in protein matrices. In conclusion, RAC is considered heat stable in water and can not be completely degraded in protein matrices likely due in part to its low protein binding capacity. The degree to which different protein affects the thermodegradation of RAC remains to be studied. Heating of RAC residues in food cannot always be assumed safe.

PS 2432 STABILIZING EFFECTS OF PH, GALACTOSE, AND EXOPOLYSACCHARIDES ON THERMAL INACTIVATION OF RICIN IN FOODS.

W. H. Tolleson[1], O. A. Triplett[1] and L. S. Jackson[2]. [1]*Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR and* [2]*National Center for Food Safety and Technology, Summit-Argo, IL.*

The thermal stability of proteinaceous toxins can be influenced by the chemical environment of foods, which vary in acidity or in the amounts and types of lipids, protein, and carbohydrates present. Ricin is a potent ribosome-inactivating protein toxin produced by the castor bean plant. In a separate study, ricin was added to a panel of foods marketed for infants and small children and the kinetics for thermal inactivation of its cytotoxic activity were measured. Those experiments showed that products containing yogurt conferred enhanced thermal stability to ricin. In studies presented here, a blended yogurt fruit drink product was selected for further study because ricin exhibited the greatest thermal stability when added to that food. Thermal inactivation of ricin in yogurt fruit drink at 75°C at its native pH 3.97 yielded a half-life of 40 ± 2 min. Thermal inactivation of ricin in this food adjusted to pH 3.5, 4.5, 5.5, 6.5, and 7.5 resulted in half-lives of 24 ± 5, 125 ± 17, 22 ± 3, 3.7 ± 0.4, and 5.4 ± 1.0 min, respectively. Galactose levels were higher in yogurt fruit drink and plain yogurt (3.8 ± 0.9 and 6.1 ± 0.1 mg/mL, respectively) than in other foods tested (≤1 mg/mL). Compared to controls, ricin in the presence of 3.8 and 18.0 mg/mL galactose exhibited 2.5- or 2.8-fold higher residual activity after 60 min incubation at 75°C. Yogurt microorganisms produce galactose-rich exopolysaccharide polymers that contribute to the viscosity and texture associated with this food. Exopolysaccharides extracted from yogurt fruit drink and reconstituted in whole milk increased the half-life of ricin at 75°C from 3.7 ± 0.5 min to 8.1 ± 0.1 min. Our study identified three characteristics of foods that influence the thermal stability of ricin and suggest that the thermal stabilities of related galactose-binding protein toxins, such as abrin and the Shiga-like toxins produced by pathogenic *Escherichia coli* O157:H7, could be influenced in an analogous manner.

PS 2433 A KINETIC STUDY OF THERMAL INACTIVATION OF THE BIOTERRORISM AGENT RICIN IN FOODS FOR INFANTS AND SMALL CHILDREN.

O. A. Triplett[1], W. H. Tolleson[1] and L. S. Jackson[2]. [1]*Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR and* [2]*National Center for Food Safety and Technology, Summit-Argo, IL.*

Ricin is a highly toxic protein produced by the castor bean plant, an agriculturally important crop (1.4 million tons of seeds harvested in 2005) cultivated as a source of castor oil and ricinoleic acid. At 1-5% ricin by weight, the 730,000 tons of castor bean wet mash produced annually for oil extraction could contain up to 36,500 tons of active toxin, if it is not treated with heat or chemical agents to render it safe. Ricin is classified as a Category B Select Agent because its toxicity and relative abundance enhance its potential for exploitation by bioterrorists. Infants and young children present vulnerable targets for food-borne bioterrorism agents because their low body weight and tendency to consume a single type of food as a complete meal (e.g. baby foods or infant formulas) may allow exposure to higher relative doses. Their vulnerability may be compounded because children's natural defense systems have not matured and because infant formula, jarred baby foods, and beverage foods for infants and small children normally do not involve further cooking at home that could inactivate biological or biochemical toxins. A panel of beverage foods and food components marketed for infants and small children was selected (whole milk, milk- and soy-based infant formulas, clarified and cloud-containing apple juices, pulp-free and pulp-containing orange juices, a blended yogurt fruit drink, and plain yogurt). Ricin was added to each food, incubated at 75°C for various time intervals, and the residual cytotoxic activity in each sample was measured using RAW264.7 macrophage cells. Ricin exhibited similar half-lives in all foods tested except yogurt fruit drink and plain yogurt, in which ricin displayed longer half-lives. Ricin half-lives in foods correlated strongest with galactose and protein levels and less with lactose levels and native pH, factors known to stabilize ricin. These data show how ricin inactivation rates can be altered by food composition.

PS 2434 OCTOPUS AS A FOOD PRODUCT: A SIGNIFICANT SOURCE OF ESSENTIAL ELEMENTS (CU AND CR) AND HUMAN EXPOSURE TO TOXIC ELEMENTS (PB).

L. Saldivar[1], T. Rodríguez-S[1], M. Castilla-M[1], M. A. Zezzi-A[2] and F. Barbosa-J[3]. [1]*Chemistry Faculty, Universidad Nacional Autónoma de México, Universitaria, D.F, Mexico,* [2]*Chemistry Institute, State University of Campinas, Campinas, Sao Paulo, Brazil and* [3]*Faculty of Pharmaceutical Sciences, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.* Sponsor: D. Acosta.

Some metals are essential for maintaining proper human health. They are nutritionally essential at low levels but toxic at high levels and adverse nutritional effects can occur if these metals are not available in sufficient amounts. There are others metals with not known biological functions considered as toxic elements. Metals can bioaccumulate in aquatic organisms and be consumed by humans, representing the food chain a risk to human health. This research evaluates the dietary exposure of the Mexico City inhabitants to essential trace elements Cu and Cr, and to the non-essential Pb. Octopus samples were collected at markets from each D.F. 16 delegations. Tentacles and heads were digested in microwave oven(HNO3 y H202). Analysis was performed by inductively coupled plasma-quadrupole mass spectrometry(ICP-QMS), using IAEA V-10 as CRM. The intervals obtained for total con-

centration(μg/g) were: 5.1–95.3; 2.4–4.8 and 0.1–1.6 for Cu, Cr and Pb, respectively. The statistical analysis shows differences between the average and median values for Cu data, and high dispersion for Cu and Pb(head, supermarket). The results were compared with the existing mexican regulations for heavy metals in food. According to the NOM-051-SCFI/SSA1-2010, the reference nutrition values(daily suggested intake, mg/day) are set for Cu and Cr as: 0.65 and 0.022, respectively. The maximum, average and median values obtained for Cu and Cr are above the regulations, regarding the consumer exposure. And maximum values for Pb, exceeds the recommendations from NOM-029-SSA1-1993 and NOM-129-SSA1-1995(maximum limit value:1.0mg/kg Pb).

PS 2435 DETERMINATION OF TOXIN CONTAMINATION IN DIETARY SUPPLEMENTS MARKETED IN GERMANY.

A. H. Heussner[1], J. Fastner[2, 1] and D. R. Dietrich[1]. [1]*Human & Environmental Toxicology, University of Konstanz, Konstanz, Germany and* [2]*Section II 3.3 - Drinking-water resources and treatment, Federal Environmental Agency, Berlin, Germany.*

Blue-green algae (Spirulina sp., Aphanizomenon flos-aquae) as well as Chlorella sp. are commonly used as dietary supplements. Whereas Spirulina and Chlorella species are mostly produced in aquaculture, A. flos aquae is naturally grown and harvested from Upper Klamath Lake, Oregon, USA. Especially cyanobacterial products have given cause for serious concern in the past, especially as adverse health effects were reported by consumers repeatedly and marketed products appeared to be contaminated with toxins. The aim of the current study was to determine the type and level of toxin contamination in dietary supplement products marketed in Germany. Thirteen products from the German market (bought randomly in 2010) and consisting of A. flos-aquae, Spirulina and Chlorella products marketed under varying brands, were analyzed for concentration levels of microcystins (MCs), nodularins, saxitoxins (PSPs), anatoxin-a and cylindrospermopsin. Additionally, five products tested previously (2003-2008) were re-analyzed for comparison. Product samples were extracted (liquid extraction) and submitted to a cytotoxicity assay (MTT) as well as to various toxin analyses: (1) Phosphatase inhibition assay (PPIA), (2) ELISA and (3) LC-MS/MS. All extracts were cytotoxic in the epithelial cells employed, thus potentially explaining the diarrhea often reported in conjunction with the consumption of these dietary supplements. All of the A. flos-aquae products tested positive for MCs, while all samples were negative for the other toxins. The contamination levels of the MC-positive samples were either below or around the guideline value of 1 μg MC-LR equivalents/g d.w. of the Oregon Department of Health. However, in view of the fact that MC-LR was classified as a probable human carcinogen (2B) and the fact that MCs were demonstrated to potentially contribute to the onset and progression of neurodegenerative diseases the observed MC contamination levels are clearly of concern.

PS 2436 SPIRULINA: TRANSITION FROM POOR MAN'S FOOD TO A DIETARY SUPPLEMENT.

J. C. Griffiths, G. I. Giancaspro and N. Sarma. *Documentary Standards, U.S. Pharmacopeia, Rockville, MD.*

Cyanobacterial organisms (commonly known as blue-green algae) had and still have a long history of use as regional food across several continents. Arthrospira platensis a.k.a Spirulina platensis and A. maxima a.k.a. S. maxima have recently gained widespread and extensive use as dietary supplements due to their high levels of quality protein with a complete array of essential amino acids, coupled with high levels of minerals, vitamins, and fatty acids such as gamma-linolenic, docosahexanoic and arachidonic. Because much of the commercial spirulina is still obtained from open tropical and subtropical lakes and ponds, and the principal growers are located in Southeast Asia and Central/South America, the resulting mass harvesting has brought about issues of contamination with other species of Cyanobacteria. This work reviews the toxicological concern from contamination with microcystin-producing non-Spirulina genera (such as Microcystis and Aphanizomenon), the issue of quality control for spirulina dietary supplements, and the safety evaluation from US Pharmacopeia for development of a spirulina quality monograph.

PS 2437 FIFTY YEARS OF CHANGE: FATE AND FORTUNE OF SELENIUM AND MERCURY.

J. C. Griffiths[1], S. A. Jordan[2] and T. L. Cecil[1]. [1]*United States Pharmacopeia, Rockville, MD and* [2]*Health Canada, Ottawa, ON, Canada.*

Fifty years ago, salts of the elements Selenium (Se, At. No. 34) and Mercury (Hg, At. No. 80) were found within the pages of the United States Pharmacopeia (USP) as bona fide pharmaceuticals…and in current editions they still are…but with a significant reversal of fortunes. In 1961 selenium sulfide was a presumed toxicant and its pharmacopeial use limited as an external-only anti-seborrheic. At that same time, there were several mercury-based salts, i.e., mercurous chloride and mercurophylline, for oral and parenteral administration, primarily as diuretics and cathartics. Fast forward to 2011…selenium is now an essential element given orally in such forms as selenomethionine and selenized yeast, and mercury in all forms is a tightly controlled and limited as a heavy metal impurity. What changed; the elements, their toxicity, or our understanding? It is obvious the elements did not change, but the field of toxicology has changed. The scientific understanding of how and in what fashion these two elements impact their respective targets has developed - in the case of Hg, central nervous system and the kidneys, in the case of Se as a trace essential in nutrition via key metabolic enzymes. The benefit:risk assessment of these elements, when, used for therapeutic purposes has changed over time. For instance, in the case of mercury, the development of alternative drugs used for similar indications, which have a much better benefit:risk profile, lead to the abandonment of this metal for many of its historical uses. Evolution of the scientific knowledge with regard to the nutritional role of selenium was a factor in its increased use in therapeutic products.

PS 2438 RISK ASSESSMENT OF CHICORY ROOT EXTRACT AS A FOOD INGREDIENT.

R. A. Matulka and G. A. Burdock. *Burdock Group, Orlando, FL.*

The root of the chicory plant (Cichorium intybus L.) has been consumed for centuries as a medicinal plant and as food, and both the root and extracts of the root have gained renewed interest for use in food and dietary supplements. Chicory root extract is obtained by the water extraction of either the raw or from roasted chicory root. Chicory has been grown commercially in the US and Europe for the production of extractable inulin, a soluble fiber gaining commercial applications in a variety of foods and dietary supplements. Additional substances found in the chicory root extract include fructose oligosaccharides and volatile substances that include furfural, oxymethylfurfural and 5-hydroxymethyl-2-furfural, as well as acetophenone. Chicory root extract has been used as a flavor enhancer and flavoring agent in many food categories, including baked goods, frozen dairy, gelatin puddings, hard and soft candy, sweet sauces, and alcoholic and nonalcoholic beverages. Per capita consumption estimates find that chicory root extract is consumed as a food ingredient at 0.085 mg/kg body weight/day. Water extracts of chicory root may be increasing consumption as a tea, while use in dietary supplements may be increasing due to potential effects on inflammation and potential fiber supplement. Acute and short-term studies conducted on chicory root extract or its constituents indicate a low level of toxicity. Subchronic (28-day) administration of chicory root extract to rats indicated no toxicity at up to 1000 mg/kg/day, while higher doses of chicory extract administered to adult male Swiss mice over a ten-day period resulted in significant toxicological effects on the reproductive system. Conflicting studies have been published on the mutagenic potential of chicory root extract, while other research indicates potential cytotoxicity of chicory root extract components. In humans, administration of chicory root extract at up to 1800 mg/day was well-tolerated. In summary, on the basis of the data presented above, consumption of chicory extract as an added food ingredient is safe at present use levels.

PS 2439 USP COMPENDIAL MONOGRAPHS FOR DIETARY SUPPLEMENTS: HELPING TO ENSURE PRODUCT QUALITY—BLACK COHOSH AS A CASE STUDY.

N. D. Sarma[1], G. I. Giancaspro[1], M. Sharaf[1], J. C. Griffiths[1], S. A. Jordan[2] and R. J. Marles[2]. [1]*United States Pharmacopeia, Rockville, MD and* [2]*Health Canada, Ottawa, ON, Canada.*

The manufacture of commercial herbal dietary supplements and natural health products is sometimes plagued with issues of adulteration, contamination, and misidentification. Black cohosh (BC, *Actaea racemosa L., Ranunculaceae*) has become a popular botanical supplement for symptomatic relief during menopause. Recent studies in the United States and Canada have shown that some commercial products labelled as containing this plant have not actually contained authentic BC. In Canada, active pharmacovigilance was able to identify several BC products, linked to adverse reactions, which did not contain authentic BC. The United States Pharmacopeia (USP) compendial monograph for BC provides a method to distinguish between authentic BC and other related plant species if used appropriately and routinely as part of the supply-chain due diligence. USP monographs for dietary supplements (including those for BC) are developed with participation from stakeholders (academic, regulatory and industry subject matter experts) and the analytical methods are validated for accuracy, precision, specificity, ruggedness and robustness. The mis-identification of BC shows that public standards from USP provide an opportunity to ensure the quality of dietary supplements. Further research

is required to determine if other reported hepatic adverse reactions associated with products labelled as containing BC may be related to mis-identified herbs present in the implicated products, or if other factors may be responsible.

PS 2440 SAFETY ASSESSMENT OF FOOD INGREDIENTS ORIGINATING FROM GENETICALLY MODIFIED BACTERIA.

L. Dolan and G. A. Burdock. *Burdock Group, Orlando, FL.*

The FDA has developed guidance for data that should be included in food additive petitions and generally recognized as safe (GRAS) notifications for enzyme preparations. While this guidance includes some information about additional requirements that are necessary for enzyme preparations from genetically modified organisms (GMOs), a specific guidance document delineating all steps and tests that are necessary for the safety assessment of enzyme preparations from genetically modified bacteria (or products derived from use of these enzymes) has not been created. The purpose of this poster is to systematically describe issues that should be addressed to obtain approval of food ingredients derived from genetically modified bacteria (GMB), and tests that may be performed to address these issues. Several GMB-derived enzymes (or products derived from them) that have undergone the series of tests mentioned in this abstract have received generally recognized as safe (GRAS) status. First and foremost, the source and production strains of the bacteria should be nonpathogenic and have a history of safe use. The vector should contain DNA sequences that will precisely code for enzyme production. Specifications for materials produced from the GMB should include limits for antibiotics (if used in production), pathogenic bacteria, and bacterial endotoxins. Tests must demonstrate that that: 1) the sequences of the synthesized and native enzymes are identical; 2) the synthesized enzyme is not allergenic; and 3) the enzyme (or the ingredient produced from the enzyme) is not contaminated with bacteria, DNA, or toxins. Conventional toxicity tests that are required for new food ingredients also should be performed in order to determine a safe level of exposure to the ingredient synthesized by the GMB. By performing the analyses and tests mentioned above, the producer should be able to demonstrate that the GMB-synthesized food ingredient would be as safe as the same ingredient produced by a conventional method.

PS 2441 APPROACH FOR DETERMINING IF DATA ON HETEROLOGOUS STRAINS OF SIMILAR SPECIES WOULD SUPPORT THE SAFETY OF A PARTICULAR STRAIN WHERE DATA ARE LACKING.

S. Choi, K. Howse and A. Roberts. *Cantox Health Sciences International, Mississauga, ON, Canada.* Sponsor: L. Haighton.

When attempting to assess the safety of a particular strain, there are often limited toxicological data for the strain of interest. In these cases, data on other strains within the same species may provide important insight into the safety of the species as a whole. We can gain insight into toxigenic, pathogenic, or allergenic properties associated with the species. This information is useful in determining if the strain is derived from a safe lineage. However, in order to use such data, it first must be determined whether the strains are sufficiently similar from a toxicological point of view. We propose a method for determining when a surrogate strain can be used to support the safety of another strain of interest. The first step is to analyze the genetic similarity of the strains (i.e., DNA-relatedness) and determine sequence differences and the genes within these regions. Differing sequences must be analyzed to determine if there are resulting changes at the phenotypic level. These type of data are useful in determining whether the strains may differ in the expression of deleterious products. The second step is the consideration of the composition of the resulting product; the major constituents of the end products should be compared to ensure that they are sufficiently similar between strains. Third, exposure of the surrogate strain must cover the expected exposure of the strain in question. Finally, potential properties of the surrogate strain, such as expression of antibiotic resistance genes, toxins, or allergens, must be assessed for safety. Using the results from the above analyses, it can be determined if data from a surrogate strain support the safety of a particular strain of interest.

PS 2442 COMPOSITION OF MULTIPLE LOTS OF EVENING PRIMROSE OIL COMPARED WITH CORN OIL.

T. Cristy[1], S. Graves[1], V. G. Robinson[2] and C. Smith[2]. [1]*Battelle Memorial Institute, Columbus, OH and* [2]*National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

Evening Primrose Oil (EPO) has been selected for testing on the National Toxicology Program due to widespread use as a dietary supplement and the lack of adequate toxicological data. Four lots of EPO and one lot of corn oil were procured from different vendors. They were analyzed for fatty acids by gas chromatography following saponification and methylation, peroxides by titration, and sterols and tocopherols by gas chromatography. All lots of EPO consisted of 67 to 71% linoleic acid, 8 to 9% γ-linolenic acid, 6% palmitic acid, 5% oleic acid, 2% stearic acid, and less than 1% each of α-linolenic, eicosenoic, and arachidic acids. The composition of the corn oil was similar with 53% linoleic acid, 10% palmitic acid, 24% oleic acid, 2% stearic acid, and less than 1 percent each of α-linolenic, γ-linolenic, eicosenoic, and arachidic acids. The only sterol or tocopherol present above 0.1 percent in the oils was β-sitosterol, which was present at approximately 0.3 percent in the EPOs and 0.2 percent in corn oil. Stigmastanol, stigmasterol, campesterol, α-tocopherol, Δ-tocopherol, and γ-tocopherol were all detected, but less than 0.1 percent. Three of the four lots of EPO had peroxide numbers (10 – 20 meq/kg) higher than the fourth (1 meq/kg), indicating they had undergone more oxidation during storage. This work was supported by NTP Contract N01-ES-55551.

PS 2443 MINERAL CONTENT OF COMMONLY INGESTED BEVERAGES AND BOREHOLE WATER IN NNEWI, NIGERIA.

O. Orisakwe[1], H. Enuh[2], N. Ekwo[2], V. Obidile[2] and A. Ibekie[2]. [1]*University of Port Harcourt, Rivers State, Port Harcourt, Nigeria and* [2]*Nnamdi Azikiwe University, Nnewi, Nigeria.*

Epidemiological studies have examined relationships between exposure to trace elements and minerals in dinking water/beverages and the occurrence of disease, including reproductive outcome. Because water-bone minerals are in ionic form and are easily absorbed by the gastrointestinal tract, it has been suggested that drinking water may be an important source of mineral intake. Using a market basket method we examined the chemical status of commonly ingested beverages including borehole (ground water) in Nnewi, Nigeria with respect to K+, Na+, Ca2+, Mg2+ and pH values and hence to ascertain the nutrient value of these elements in water using Flame photometry.The pH ranged from 6.27-6.88 in Otolo, 6.40- 7.30 in Uruagu, 6.68-7.50 in Nnewiichi and 6.01-6.69. Na+ and K+(mg/dl) ranged from 2.30-13.30 and 3.51-6.24 respectively. Chivita Orange Juice had highest level 828mg/dl of Na+ and SQUAD 5 had 780.0 mg/dl of K+. Ca2+ and Mg2+ were highest in Vita Vita Apple Drink (113.6mg/dl) and Maltina Sip-it (34.51mg/dl) respectively. Imported beverages had minerals ranged from 46-690 mg/dl (Na+), 19.5-780mg/dl (K+), 40.8-136.4mg/dl (Ca2+) and 12.64 – 23.57mg/dl of Mg2+ . The results suggest that borehole water and beverages may be important dietary sources of Ca2+, Mg2+, Na+ and K+. We may conclude that borehole water and beverages may be important dietary sources of Ca2+, Mg2+, Na+ and K+. Drinking water sources and beverages available to the local population in Nnewi, South Eastern Nigeria especially Nnewi may contain clinically important levels of these of minerals. This research was made possible by funding from The Africa Education Initiative (www.nef3.org)

S 2444 OVERVIEW.

W. Klimecki. *University of Arizona, Tucson, AZ.*

Autophagy is a cellular process by which organelles, cytoplasm, and specific proteins are delivered to the lysosome where they are degraded. Autophagy has been described for at least four decades, but the scope and the importance of this programmed cellular response have only recently come into focus, heralded by an exponential increase in autophagy publications. One clear message that is emerging from these early studies of autophagy is its relevance to the field of toxicology. Diverse xenobiotics are capable of perturbing autophagy, with effects ranging from autophagy induction to complete inhibition. The functional consequences of these perturbations are complex: in some instances autophagy induction is cytoprotective, however in some instances its induction is a cell death pathway. The significance of autophagy to toxicology is underscored by its fundamental role in biology. It is an ancient process. Many autophagy pathway members were initially characterized in Saccharomyces cerevisiae, where autophagy can be induced by nutrient deprivation. In Drosophila melanogaster autophagy plays a critical role in embryonic development, responding to steroid signaling pathways by executing programmed cell death. In species from Caenorhabditis elegans to Homo sapiens autophagy plays a key role in mitochondrial dynamics and in clearing damaged mitochondria. In the immune system autophagy is critical to antigen presentation, the development of regulatory and effector functions, and the acquisition of tolerance. Thus, autophagy plays an evolutionarily conserved role in multiple biological processes. The ability of environmental stress and xenobiotics such as inorganic arsenic to modulate autophagy points to the need for an appreciation of this long-known, but poorly understood, pathway in toxicology.

S 2445 AUTOPHAGY IN DEVELOPMENT: A LIFE OR DEATH DECISION.

E. Baehrecke. *Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA.* Sponsor: W. Klimecki.

Embryonic development involves a tightly controlled balance between steroid signaling, cell growth arrest, and autophagic cell death. Autophagy has been implicated in both cell survival and cell death, and it is important to resolve how this catabolic process can function in such distinct cell fates. Our studies focus on steroid-activated autophagic cell death during development of *Drosophila*, using larval salivary glands as a model. An increase in steroid level triggers nearly synchronous cell death in salivary glands. Interestingly, steroid levels rise and fall at multiple stages during development, but salivary glands always die in response to a rise in hormone at a specific stage that follows cell growth arrest. This developmentally-regulated cell death utilizes apoptosis genes, including caspases, but neither caspase inhibition nor mutating caspase genes completely inhibit salivary gland degradation. Salivary glands possess the morphology of cells that die by autophagic cell death, and autophagy is required for their complete degradation. Because autophagy can, in different contexts, be either a compensatory mechanism aimed at cell survival or a cell death effector, it is important to understand the factors that regulate and may distinguish the use of autophagy in cell survival and cell death.

S 2446 AUTOPHAGY IN IMMUNITY AND INFLAMMATION.

V. Deretic. *Department of Molecular Genetics and Microbiology, University of New Mexico, Albuquerque, NM.* Sponsor: W. Klimecki.

Autophagy is a fundamental biological process that enables cells to autodigest portions of their own cytosol, with a wide spectrum of roles including aging, development, neurodegeneration, and cancer. The immunological role of autophagy was first recognized for the ability of autophagy to sanitize the cellular interior by killing intracellular microbes. Since then, the repertoire of autophagy roles in immunity has been vastly expanded to include a diverse but linked portfolio of regulatory and effector functions. Autophagy is an effector of Th1/Th2 polarization, it fuels MHC II presentation of cytosolic (self and microbial) antigens, shapes central tolerance, affects B and T cell homeostasis, acts both as an effector and a regulator of Toll-like receptor and other innate immunity receptor signaling, and contributes to chronic inflammatory disease in human populations. With such a multitude of innate and adaptive immunity functions, the study of the immunological roles of autophagy has opened new frontiers in contemporary immunology research. This presentation will introduce the fundamentals of autophagy, focusing on the maze of different immunological aspects of autophagy, particularly the role of autophagy in infectious and chronic inflammatory diseases.

S 2447 ARSENIC, AUTOPHAGY, AND IMMUNOTOXICITY.

W. Klimecki, R. M. Douglas, F. Zhao and A. M. Bolt. *Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.*

Exposure to air, water and food contaminated by inorganic arsenic is a global problem that has resulted in substantial morbidity and mortality. Among several systemic targets of arsenic in humans is the immune system. Epidemiological studies have documented increased morbidity from infectious disease as well as decreased neonatal thymic size related to elevated drinking-water arsenic concentration. These findings are supported by *in vitro* and *ex vivo* studies. We are using human lymphoblastoid cell lines (LCL) as models of immunological toxicity of environmental arsenic exposure to human populations. LCL exposed to cytotoxic levels of arsenite (6 uM) demonstrate hallmarks of autophagy, including the appearance of autophagosomes and autolysosomes in electron micrographs, an increase in cellular fluorescent labeling of lysosomes, and an increase in levels of LC3-II protein. The ensuing cell death occurs without any evidence of apoptosis. LCL exposed to a lower, more environmentally relevant arsenite concentration (0.75 uM) also undergo autophagy induction accompanied by an inhibition of cell proliferation, an effect consistent with other studies of arsenic immunotoxicity. Accompanying this proliferative inhibition are gene expression changes consistent with the endoplasmic reticulum stress response, and a coordinated induction of genes whose products are localized to the lysosome. The pathways targeted by arsenite in LCL have well-defined roles in normal immune function. The use of LCL as models of arsenic's effect on immune cells is shedding light on a potential role for autophagy in arsenic-induced immunotoxicity.

S 2448 THE ROLES OF MITOCHONDRIAL FUSION, FISSION, AND AUTOPHAGY IN REMOVING DAMAGED MITOCHONDRIAL DNA.

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

The importance of mitochondrial fusion, fission, and autophagy in mitochondrial health is increasingly clear. We have found that these processes play an important role in the maintenance of mitochondrial DNA (mtDNA) integrity. Helix-distorting adducts formed after exposure to environmentally important genotoxins such as ultraviolet C (UVC) radiation and some polycyclic aromatic hydrocarbons and mycotoxins are repaired in the nuclear genome by nucleotide excision repair. However, nucleotide excision repair proteins are not present in the mitochondria, and some of these same genotoxins also cause more damage to the mitochondrial than nuclear genome. Thus, mtDNA is especially vulnerable to such genotoxins. Therefore, we carried out experiments to test the fate and effects of such damage in the mitochondrial genome. Using the nematode model *Caenorhabditis elegans*, we found that UVC-induced photodimers present in mtDNA are removed slowly, and that this process depends at least in part on mitochondrial fusion, fission and autophagy. Further, persistence of this damage leads to decreased ATP levels, larval developmental arrest, and neurodegeneration. Mutations in mitochondrial fusion and autophagy genes exacerbate the larval arrest, suggesting a potent gene-environment interaction in which the effects of mtDNA damage caused by environmental agents are exacerbated by decreased mitochondrial fusion and autophagy. This research was supported by funding from the National Institutes of Environmental Health Sciences, 1 P30 ES-011961-01A1 and National Institute of Neurological Disorders and Stroke, 1 R21-NS065468-01.

S 2449 MITOPHAGY AS A MECHANISM OF MITOCHONDRIAL TURNOVER AND QUALITY CONTROL.

J. J. Lemasters. *Pharmaceutical & Biomedical Sciences and Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC.*

Reactive oxygen species are a by-product of mitochondrial respiration that damage mitochondria and mitochondrial DNA. Mitochondrial damage also frequently contributes to pathogenesis in disease processes and after toxic stresses. Mitochondrial autophagy, or mitophagy, is the major degradative pathway in mitochondrial turnover. Long assumed to be a random process, increasing evidence indicates that mitophagy is selective for damaged mitochondria. This presentation will give an overview of the process of mitophagy, the possible role of the mitochondrial permeability transition in mitophagy, and the importance of mitophagy for elimination of dysfunctional mitochondria.

S 2450 HUMAN PLURIPOTENT STEM CELLS AND NEURAL PROGENITORS AS MODELS OF GENE-ENVIRONMENT INTERACTIONS IN NEUROLOGICAL DISEASE.

A. B. Bowman[1] and P. J. Lein[2]. [1]*Neurology, Vanderbilt University, Nashville, TN and* [2]*Molecular Biosciences, University of California Davis School of Veterinary Medicine, Davis, CA.*

A complex relationship of environmental and genetic risk factors underlies many neurodevelopmental and neurodegenerative diseases, yet identification of causative factors has been severely hampered by the lack of rigorous experimental models that incorporate the combinatorial influence of diverse toxicants and the inherent genetic variation in human susceptibility and exposure. This complexity and the likelihood that the balance of genetic and environmental influences varies tremendously between individuals also complicate epidemiological studies aimed at identifying environmental risk factors for specific neurological diseases. The increasing availability and characterization of human neuroprogenitor cells has accelerated the development of models for high- throughput screening for chemicals with potential adverse effects on the developing human nervous system. The more recent development of induced pluripotent stem cell (iPSC) technology now permits the establishment of differentiated in vitro cultures of patient-specific neurons to assess susceptibility to neurotoxicants. The advantage of this approach is that environmental risk may be evaluated without a priori knowledge of an individual's genetic risk factors. A number of technical and theoretical hurdles need to be overcome before the full potential of these approaches can be realized. Thus it is important to highlight the newest research by experts in this burgeoning field to frame the possibilities of iPSC technology and human neural progenitors for the study of genetic and environmental risk factors that influence the susceptibility to

and progression of neurological diseases. In conclusion, the potential human iPSC approaches offer for translational toxicology and assessment of human risk factors will be discussed.

S 2451 APPLICATIONS OF HUMAN STEM CELL TECHNOLOGY TO NEUROTOXICOLOGY.

P. J. Lein. *Veterinary Medicine, Molecular Biosciences, University of California Davis, Davis, CA.*

Brief opening remarks will introduce the audience to the topic of human pluripotent stem cells and induced pluripotent stem cells (iPSCs) and provide an overview of the technical challenges and exciting opportunities for neurotoxicology provided by this evolving technology.

S 2452 USING NEURAL PROGENITOR CELLS IN HIGH-THROUGHPUT SCREENS FOR DEVELOPMENTAL NEUROTOXICANTS: TRIUMPHS AND TRAGEDIES.

T. J. Shafer, M. Culbreth, J. M. Breier and W. R. Mundy. *Integrated Systems Toxicology, U.S. EPA, Research Triangle Park, NC.*

Current protocols for developmental neurotoxicity testing are insufficient to test thousands of commercial chemicals. Thus, development of high-throughput screens (HTS) to detect and prioritize chemicals that may cause developmental neurotoxicity is needed to improve protection of public health. While a variety of cell culture models are amenable to this approach, neuroprogenitor (NP) cells, particularly those of human origin, may provide highly relevant models for developmental neurotoxicity testing. Over the past 5 years, we have examined the utility of different NP cell models for HTS assays of proliferation, apoptosis, viability and differentiation. Using ReNcell CX cells, a human cortical NP cell, we measured changes in proliferation (BrdU incorporation) induced by known antiproliferative compounds as well as compounds (0.001-100 μM) known to cause developmental neurotoxicity; non-neurotoxic compounds were without effect. Furthermore, we demonstrated that this approach is amenable to HTS by screening a set of 309 chemicals for effects on proliferation. More recently, we demonstrated that ReNcell CX cells are also useful for HTS assays for apoptosis (activated p53 and Caspase) by identifying appropriate assay positive controls. We have conducted more limited assessment of proliferation in other human NP cells. Recently, we compared ReNcell CX cells to mouse cortical NP cells and demonstrated that both cell types are sensitive to compounds known to alter proliferation and/or apoptosis. Development of HTS assays for differentiation has been more challenging. NP cells tested to date have not differentiated rapidly, and it has been difficult to identify a cellular marker for committed neurons suitable for this endpoint. Our work demonstrates that NP cells can be utilized in HTS assays for the important neurodevelopmental endpoints of proliferation and apoptosis, but more development will be needed to use these cells in assays for neuroprogenitor differentiation. (This abstract does not reflect Agency Policy)

S 2453 TRENDS OF THE IPSC TECHNOLOGY FOR BIOMEDICAL RESEARCH AND CELL THERAPY: POTENTIAL OF PROTEIN-INDUCED IPSCS.

K. Kim. *McLean Hospital/Harvard Medical School, Belmont, MA.* Sponsor: A. Bowman.

Widely established methods to generate induced pluripotent stem cells (iPSCs) require the use of viral and/or genetic materials that likely integrate into the chromosomal DNAs with unknown genetic changes. The Kim lab has developed a method to generate stable iPSCs from human fibroblasts by directly delivering four reprogramming proteins (Oct4, Sox2, Klf4, and c-Myc) fused with a cell-penetrating peptide (CPP). Approaches such as these have the potential to decrease confounding variability in iPSC outcome measures due to the use of viral and chemical mediators of reprogramming that have the potential to permanently influence gene expression, neuronal differentiation and toxicant sensitivities. In support of this, we found that precursor cells derived from lentivirus- and retrovirus-based human iPSCs exhibit early senescence and significant apoptotic cell death whereas those from protein-based human iPSCs and human ESCs do not show such abnormal phenotypes. This presentation will discuss our comparative studies of neural differentiation and their cellular and molecular characteristics using human iPSC lines that are generated by different methods. These studies will enable the design of the robust methodology and quality control of individual iPSC lines that is necessary for eventual high throughput analysis of gene-environment interactions across patient-derived iPSC lines.

S 2454 *IN VITRO* MODELS OF ANGELMAN AND PRADER-WILLI SYNDROME *VIA* INDUCED PLURIPOTENT STEM CELL TECHNOLOGY.

M. Lalande. *Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT.*

The recent discovery of nuclear reprogramming of human somatic cells into induced pluripotent stem (iPS) cells offers an innovative and relevant approach to study human genetic and neurogenetic diseases such as Angelman syndrome (AS) and Prader-Willi syndrome (PWS). We have reprogrammed AS and PWS patient dermal fibroblasts using the four Yamanaka factors (POU5F1 [OCT4], SOX2, MYC and KLF4) and LIN28 delivered to the cells with retroviral vectors. Using this approach, we have obtained iPS cell lines for several different patients and differentiated these in vitro into neurons using a protocol that closely mimics human neuronal development. We are performing immunocytochemistry to determine the developmental timing of expression of various neuronal markers during differentiation from both the normal and patient iPS cells and measuring action potentials and excitatory post-synaptic potentials from these neurons at later times (10 weeks) of differentiation. Moreover, RNA is being extracted from patient-specific and normal iPS cells and iPS-derived neurons to produce strand-specific cDNA libraries for whole transcriptpme (RNA-seq) analysis in order to identify disease-specific gene networks. Our goal is to model the effects of these specific human gene defects in vitro in order to study the disease mechanisms and regulation of genomic imprinting in AS and PWS. The longer term goal of this work is to test small molecules, environmental modulators, and potential therapies in the human neuronal cell culture models of AS and PWS.

S 2455 PATIENT-DERIVED STEM CELLS AS A TRANSLATIONAL MODEL FOR NEUROTOXICOLOGICAL RISK.

A. B. Bowman. *Neurology, Vanderbilt University, Nashville, TN.*

The Bowman lab is developing methods to test the sensitivity of neurons differentiated from patient-specific induced pluripotent stem cells (iPSCs) to neurotoxicants associated with neurodegenerative disease. We have generated human iPSCs from patients having various neurological diseases (e.g. Parkinson's disease and Tuberous sclerosis) and controls. These human iPSCs have been differentiated into neuronal progenitors (evaluated by expression of PAX6 and other neural precursor markers). These progenitors have subsequently been patterned to specific neuronal subtypes including dopaminergic (Tryosine Hydroxylase positive) neurons. We have successfully adapted these neuronal differentiation methods to 96-well plate format to enable mid to high-throughput gene expression marker analysis by quantitative RT-PCR as well as cell survival assays for neurotoxicity testing. Environmentally relevant metal neurotoxicants are being tested for their effect on these differentiated human neuronal cells, including manganese chloride (50μM to 1000μM) and methyl mercury (100 nM to 10 μM). For example, manganese exposure (500μM, 20 hours) resulted in complete loss of neuronal processes in human dopaminergic neurons. Results from these ongoing studies will be presented. Finally, approaches for efficient neuronal differentiation and toxicological assessment methodology will be presented – with emphasis on the potential of patient specific toxicological risk assessment with human iPSCs.

S 2456 THE USE OF EPIDEMIOLOGICAL DATA AND PBPK MODELING IN A RISK ASSESSMENT: MANGANESE AS A CASE STUDY.

H. J. Clewell[1], M. Andersen[1], V. Tait[2], H. Roels[3] and W. Boyes[4]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC,* [2]*University of Ottawa, Ottawa, ON, Canada,* [3]*Université Catholique de Louvain, Brussels, Belgium and* [4]*U.S. EPA, Research Triangle Park, NC.*

Manganese (Mn) is an essential element that is neurotoxic at high exposures. In recent years there has been increasing use of PBPK modeling in quantitative risk assessments to support animal-to-human extrapolation when human risk estimates are based on animal studies. However, any risk assessment for the neurological effects of Mn would likely be based on epidemiological data from occupational expo-

sures to inhaled Mn in dust. Nevertheless, the risk assessment for manganese (Mn) provides an opportunity to apply PBPK modeling in a different aspect of quantitative risk assessment: replacing default uncertainty factors with chemical-specific adjustment factors. The critical issues in a Mn risk assessment based on occupational epidemiology data are the nature and extent of the uncertainty factors that should be applied to account for uncertainties regarding interindividual variability, susceptible sub-populations, duration of exposure, and different forms of Mn. We will describe the epidemiological evidence for the neurotoxic effects of Mn and explore the use of PBPK models for Mn in adult and perinatal animals and humans to predict the effect of key sources of uncertainty on the target tissue Mn dose, elucidate the dose-dependent mode of action for the neurological effects of Mn, and quantify the impact of human variability, life-stage, and other uncertainties on risks estimated from occupational epidemiology studies.

S 2457 COLLECTION OF CHEMICAL-SPECIFIC TOXICOLOGICAL AND PHARMACOKINETIC DATA TO IMPROVE RISK ASSESSMENTS BASED ON EPIDEMIOLOGY: EXAMPLE OF MN.

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

Data limitations led to the application of default uncertainty factors in a prior risk assessment for Mn. These limitations were instrumental in the EPA generation of an alternative tier II test rule under section 211(b) of the Clean Air Act, (fuels and fuel additives) regarding the proposed use a Mn-containing fuel additive (MMT). Development of both data and pharmacokinetic (PBPK) models of Mn in multiple species via oral and inhalation exposure and across ages can provide a biologically-informed basis for future risk assessment. Under the premise that target tissue dose is the key determinant of toxicity, the concentration of Mn in the brain can be estimated by PBPK models for a variety of conditions relevant to risk assessment. In addition, incorporation of specific biological mechanisms that control tissue disposition of Mn as an essential nutrient improves the biological plausibility of these models. The predicted levels from the PBPK models can be assessed using recent test rule data, in addition to other literature values. The PBPK models have the capability to consider Mn input by both oral and inhalation routes of exposure in a single evaluation, and resolve apparent route-dependent differences in toxicity. Species sensitivity differences are more complex and likely involve both pharmacodynamic and pharmacokinetic factors. Together this set of pharmacokinetic data and new PBPK models provide capabilities for comprehensive biologically based risk assessments of Mn that were not previously feasible. This abstract does not reflect EPA policy

S 2458 BIOMARKERS OF EXPOSURE TO MANGANESE AND PROSPECTIVE STUDIES OF ITS NEUROTOXIC EFFECTS IN OCCUPATIONAL COHORTS.

H. A. Roels. *Louvain Centre of Toxicology and Applied Pharmacology, Université catholique de Louvain, Brussels, Belgium.* Sponsor: H. Clewell.

Biomarkers of exposure to manganese (Mn) and neurotoxic effects of inhaled Mn are still in the focus of prevention strategies for Mn-related parkinsonian signs and symptoms in workers.
Mn in whole blood or urine has been shown to relate inconsistently to Mn in air (MnA). Our recent study in welders (n=27) showed on the first working day of the week (after 64 h without exposure) an average MnA of 26 $\mu g/m^3$ (1.3-729) and a mean Mn in plasma (MnP) of 2.1 $\mu g/L$ (1.6-4.2) at the end of the shift (controls: 1-1.9 $\mu g/L$). There was a strong MnP/MnA correlation when MnA exceeded 10 $\mu g/m^3$ ($r=0.69$, $p<0.001$, $n=20$). A MnP of 2 $\mu g/L$ can pick up welders exposed to MnA >20 $\mu g/m^3$ with a specificity of 82% and a sensitivity of 69%.
A recent prospective study in our Bay Bridge welder cohort (n=26) showed after 3.5 years of cessation of confined space welding significant improvement for cognitive function, whereas olfactory, extrapyramidal, and mood disturbances either remained status quo or exacerbated. At the time of active welding, CAL-OSHA's TLV for Mn (200 $\mu g/m^3$) was exceeded with a frequency of 55%. A separate and lower TLV for exposures to respirable Mn particulate is needed.
We also conducted a longitudinal study in workers of a Duracell battery plant in Belgium chronically exposed to airborne Mn dust (average MnA=1 mg/m^3, total dust; aerodynamic diameter <30 μm). At baseline, this cohort (n=92) showed early neurological effects in 20-30% of the subjects. Three sub-cohorts were identified for the prospective analysis based on their past chronic exposures (low, medium, high). MnA over the 8-year follow-up period showed a decreasing trend. There did not appear any trend toward worsening of the precision of eye-hand coordination (PEHC), however, a complete reversibility of PEHC was seen in the low exposure sub-cohort, while the PEHC deficit only partially improved in the two other sub-cohorts. There was no improvement in hand steadiness (postural tremor) and visual reaction time despite the decrease in Mn exposure.

S 2459 THE USE OF PBPK MODELS FOR MONKEY AND HUMAN TO INVESTIGATE THE DOSE-RESPONSE FOR THE NEUROLOGICAL EFFECTS OF MN.

H. J. Clewell[1], J. D. Schroeter[1], M. Yoon[1], A. Nong[1], M. D. Taylor[2] and M. E. Andersen[1]. [1]*The Hamner Institutes for Health Sciences, Research Triangle Park, NC and* [2]*Afton Chemical, Richmond, VA.*

A previous analysis of the dose-response for the effects of Mn in experimental animals (Gwiazda et al., 2007) relied on estimated cumulative intake of Mn as the only measure used for comparison across studies with different doses, durations, and exposure routes. The present analysis utilizes alternative dose metrics, including estimated daily uptake, estimated brain concentration of Mn, and estimated free Mn concentrations within the brain. The latter two metrics were estimated using a PBPK model that accurately accounted for the dose dependencies of Mn distribution in the monkey for combined inhalation and dietary exposures. Non-human primate response studies evaluated in the analysis included exposures by inhalation, oral, intraperitoneal and subcutaneous dose routes, and spanned durations from 23 days to 2 years. Our analysis demonstrated (1) that the use of the PBPK models makes it possible to provide a consistent description of the dose-response for the effects of Mn independent of exposure route and (2) that there is a clearly definable dose-dependent transition from reversible effects to irreversible, cumulative effects as exposures are increased. Our analysis of the various monkey studies with Mn provides further evidence of a dose-dependent transition in the mode of action for the neurological effects of Mn that needs to be considered in risk assessments for this essential metal and supports the use of tissue Mn or cumulative tissue Mn as the appropriate dose metric for these comparisons.

S 2460 APPLICATION OF PBPK MODELING TO EVALUATE SAFE EXPOSURES TO A TOXIC BUT ESSENTIAL METAL.

M. E. Andersen. *Computational Biology, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Current risk assessment methods are incapable of quantitatively considering the impact of homeostatic control in the case of systemic toxicity from inhalation of an essential element such as Mn. Novel risk assessment paradigms are required to establish consistent exposure guidelines for essential elements independent of the route of exposure. A potential strategy for devising a consistent standard-setting approach for potentially toxic essential nutrients is to restrict exposures by any route to levels that cause a minimal increase in target tissue concentrations compared to the variation of target tissue concentrations in normal individuals. Such an approach is currently being considered for the case of neurotoxicity from inhaled or ingested Mn. PBPK models of Mn have been developed to predict concentrations of Mn in the brain for both inhalation and oral exposures to Mn, as a function of the amount of Mn present in the diet. These models can also be used to estimate chemical-specific adjustment factors (CSAFs) in place of default uncertainty factors. We believe the strategy being applied to Mn is appropriate not only for Mn but also for other nutrients that are toxic in overdose conditions.

S 2461 HAZARD ASSESSMENT FOR THE ESSENTIAL ELEMENT MANGANESE BASED ON TOXICOLOGICAL, EPIDEMIOLOGICAL, AND MECHANISTIC DATA: SUSCEPTIBLE SUB-POPULATIONS AND INTER-INDIVIDUAL VARIABILITY.

V. Tait[1, 2], F. Salehi[2], M. Croteau[2], N. Karyakina[1, 2], N. Shilnikova[2], A. Ngom[2] and D. Krewski[2, 1]. [1]*Risk Sciences International Inc., Ottawa, ON, Canada and* [2]*University of Ottawa, Ottawa, ON, Canada.* Sponsor: M. Andersen.

Manganese (Mn) is an essential trace metal found naturally in rocks, soil, water and foodstuffs that is necessary for a variety of metabolic processes including bone development, nervous system function and the production and activity of a variety of enzymes. Occupational exposure to manganese occurs mainly by inhalation.

Humans are also exposed to manganese by inhalation and orally through food and drinking water; manganese deficiency is rare. An assessment of the current scientific evidence for potential health effects associated with exposure to inorganic Mn compounds has been conducted. The literature review targeted primary studies of health effects, toxicokinetics, and mechanisms of action published between Jan 1, 2005, and July 31, 2010, searching the bibliographic databases MEDLINE/PubMed, TOXLINE, EMBASE, and the HuGENet literature database. Studies published prior to 2005 were identified using secondary sources. The identification, selection, and quality evaluation of studies employed systematic review methods to avoid bias. The assessment adopts a weight of evidence approach to consider hazard to human health with respect to carcinogenicity, mutagenicity, reproductive and developmental toxicity, pulmonary toxicity, neurotoxicity and other repeated dose endpoints. For the critical neurotoxicity endpoint, this presentation also discusses: integration of new tissue dosimetry information based on recently developed pharmacokinetic models for Mn; available data on possible modifiers of risk such as iron deficiency, genetic variants, and age; recent information on mechanisms of action; and identifies data gaps that should be filled to strengthen the scientific basis on which assessments of the potential health risks of Mn may be based.

W 2462 ADVANCING PREDICTIVE ECOTOXICOLOGY TESTING AND ENVIRONMENTAL RISK ASSESSMENT IN THE 21ST CENTURY.

M. Embry[1] and D. Volz[2]. [1]*ILSI HESI, Washington, DC and* [2]*University of South Carolina, Columbia, SC.*

Following the publication of the National Research Council (NRC) Report on Toxicity Testing in the 21st Century: A Vision and a Strategy increased attention has been given to the development and use of new technologies and methods for toxicity testing. It is hoped that these new approaches will aid in the design of a routine testing strategy that will provide data that are applicable to the broadest possible range of chemicals, endpoints, and lifestages, while also providing greater detail concerning mode of action and dose/concentration-response, and reducing the overall costs, animal use, and time spent on testing. This Tox21 vision has impacted testing approaches for not only human health, but also ecological risk assessment. Therefore it is important to address these current initiatives aimed at advancing regulatory ecotoxity testing strategies. Novel approaches that could be integrated into an intelligent, tiered ecotoxicity testing strategy are under development. Additionally, concepts for utilizing chemical mode of action and/or adverse outcome pathways as a basis for developing 21st century test methods and associated predictive tools, initially developed as part of a SETAC Pellston workshop, have been expanded on several fronts. Advances in the application of QSAR and modeling approaches, cell-based assays, and 'omics methodologies as well as recent OECD efforts to develop a fish testing framework will be highlighted. Our focus will be to discuss approaches to coordinate the development of new human health and environmental toxicity testing strategies, share key lessons and advances and create effective partnerships between human health and ecotoxicology communities.

W 2463 ADVERSE OUTCOME PATHWAYS AND SYSTEMS BIOLOGY AS CONCEPTUAL APPROACHES TO SUPPORT DEVELOPMENT OF 21ST CENTURY TEST METHODS AND EXTRAPOLATION TOOLS.

D. L. Villeneuve[1], M. R. Embry[2] and D. C. Volz[3]. [1]*U.S. EPA Mid-Continent Ecology Division, Duluth, MN,* [2]*ILSI Health and Environmental Sciences Institute, Washington, DC and* [3]*Department of Environmental Health Sciences, University of South Carolina, Columbia, SC.*

The proposed paradigm for "Toxicity Testing in the 21st Century" supports the development of mechanistically-based, high-throughput in vitro assays as a potential cost effective and scientifically-sound alternative to some whole animal hazard testing. To accomplish this long-term goal, it is necessary to (1) identify and catalog common adverse outcome pathways (AOPs) and (2) based on these pathways, strategically develop a focused battery of assays with proven predictive value. The concept of AOPs provides an organizing framework for linking cellular-level responses to endpoints conventionally considered in ecological risk assessment (e.g., effects on survival, growth/development, and reproduction). Defining and cataloging AOPs provides a scientific foundation for development of toxicity pathway assays with predictive power. Systems biology is the scientific study of dynamic interactions among elements that comprise biological systems. The goal of systems biology is to understand and predict emergent properties of these complex systems. The approaches of systems biology can be used to understand the intersections and interactions among AOPs, in the context of relevant homeostatic and allostatic functions that modulate outcomes as a function of stressor intensity (e.g., dose), exposure duration, and other toxicologically relevant variables. Thus, systems biology can (1) help optimize an AOP-based in vitro testing framework and (2) aid the development of toxicodynamic extrapolation models needed for quantitative risk assessments. This presentation will highlight the utility of these two conceptual approaches, AOPs and systems biology, in evolving strategies for 21st century toxicity testing, using examples and products from recent international expert workshops with participants from academia, industry, government, and nongovernmental organizations.

W 2464 ADVANCES AND OUTLOOKS FOR QSARS IN ECOTOXICOLOGY.

S. A. Villalobos[1], S. E. Belanger[2] and P. Ranslow[3]. [1]*Nalco, Naperville, IL,* [2]*Procter & Gamble, Cincinnati, OH and* [3]*Consortium for Environmental Risk Management, Hallowell, ME.*

For the successful regulation of chemicals in commerce, authorities typically require reliable data on the effects and fate of such chemicals in humans and the environment. The norm is to provide this information using well-defined protocols for a variety of endpoints. Unlike the markets for therapeutic drugs, biocides and pesticide agrochemicals that require data rich registration packages prior to commercialization, the registration packages for industrial chemicals have been historically data poor. In its broadest definition, Quantitative Structure-Activity Relationships (QSARs) are algorithms used to predict specific properties directly from the chemical structure and without experimental testing. QSARs are used to estimate toxic effect (even exposure) concentrations, physical and/or chemical properties, environmental fate, etc. QSARs are increasingly viewed as a cost effective way to estimate ecological and health effects of chemicals by regulatory agencies around the world but also by industry, academia, nongovernment and private organizations. QSARs also play a vital role as part of the overall 3R's strategy on animal testing (Replace, Reduce, Refine). They can be a tool to evaluate adequacy of data and are useful in cases where data availability is incomplete and/or when there are differences in test methods (e.g., OECD versus USEPA guidelines). The technology behind QSARs has had such improvements that historical conservative views of their use in environmental risk assessments can now be mitigated and a reliable degree of certainty is obtainable. In spite of advances, most of the current use by regulatory agencies remains on priority setting for existing chemicals and assessments for new chemicals. This talk will highlight the most recent advancements in QSAR modeling in ecological risk assessment as well as areas where improvement is needed, including prediction of chronic toxicity, use with inorganic chemicals, mixtures, and increased regulatory acceptance.

W 2465 FISH EMBRYO AND CELL LINE ASSAYS AS POTENTIAL ALTERNATIVES FOR FISH TOXICITY TESTS.

K. Schirmer[1], K. Tanneberger[1], M. Knöbel[1, 3], N. Kramer[2], J. Hermens[2], S. Scholz[3], N. Bols[4], L. Lee[5] and C. Hafner[6]. [1]*Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland,* [2]*Institute of Risk Assessment Sciences, University of Utrecht, Utrecht, Netherlands,* [3]*UFZ, Helholtz Centre for Environmental Research, Leipzig, Germany,* [4]*University of Waterloo, Waterloo, ON, Canada,* [5]*Wilfrid Laurier University, Waterloo, ON, Canada and* [6]*Hydrotox GmbH, Freiburg, Germany.* Sponsor: M. Embry.

Fish are the most frequently used vertebrates in regulatory ecotoxicology. As vertebrates, they are legally protected animals. Therefore, alternatives to reduce or replace fish tests for risk assessment of chemicals and industrial effluents are of high societal importance. In addition to being more ethical and economical, alternative approaches may also offer better insights into the modes of action underlying the toxicity of chemicals to fish. The goal of the CEllSens project is to improve fish embryo and fish cell line assays to achieve international acceptance as alternatives to the acute fish toxicity test, which uses death as an integrative but crude endpoint. The hypothesis is that acute toxicity is in most cases caused by a non-specific interference of the chemicals with cell membranes, which are also presented as a target in fish embryo/cells. Indeed, toxicity correlation analysis between fish acute toxicity and fish embryo or cell line data revealed a very good agreement for non-specific, directly acting chemicals. However, it also became evident that compounds with high volatility and hydrophobicity are under-predicted in their toxicity in the small-scale alternative assays unless exposure concentrations are carefully verified. Moreover, specific modes of toxic action and the metabolic capabilities need to be understood; indeed lack or insufficient activity of specific metabolizing enzymes in cells and embryos was one cause for outliers from the in vivo-in vitro correlation. We suggest that dosing methods need to be developed that provide stable exposure concentrations in the small scale assays and that systems biology approaches could be a key to understand the functional capabilities of alternative experimental models to respond to chemical exposure in general.

W 2466 CRITICAL EVALUATION OF ANIMAL ALTERNATIVE TESTS FOR THE IDENTIFICATION OF ENDOCRINE ACTIVE SUBSTANCES.

F. Busquet[1], S. Belanger[2], R. Davi[3], B. Demeneix[4], J. Denny[5], M. Embry[6], M. Léonard[7], M. McMaster[8], L. Ortego[9], P. Renner[10], D. Villeneuve[5] and S. Scholz[10]. *[1]JRC, IHCP, ECVAM, European Commission, Ispra, Italy, [2]Procter & Gamble, Cincinnati, OH, [3]ExxonMobil, Annandale, NJ, [4]Muséum National d'Histoire Naturelle, CNRS, Paris, France, [5]U.S. EPA, Duluth, MN, [6]ILSI HESI, Washington, DC, [7]L'Oréal Recherche Avancée, Aulnay sous Bois, France, [8]Environment Canada, Burlington, ON, Canada, [9]Bayer CropScience, Research Triangle Park, NC and [10]UFZ, Leipzig, Germany.*

In the past 20 years considerable progress in animal alternatives accompanied by advances in the toxicological sciences and new emphases on aquatic vertebrates has occurred. A significant amount of current research is targeted to evaluate alternative test methods that may reduce, replace or refine (3Rs) the use of animals, while ensuring human and environmental health and safety. In 2009, the US EPA began implementation of the Endocrine Disruptor Screening Program which includes Tier 1 screening assays in fish and frog species which are closely aligned with the OECD test guideline series 229 and 231. However, these assays use a large number of animals and are quite long in duration relative to an ideal screening assay. As the Tier 1 assays screen and prioritize a large number of chemicals for possible endocrine activity shorter-term tests would be advantageous. In order to identify potential alternatives, a literature search was conducted and a database with alternatives to fish and frog tests testing methodologies assembled. Data from 1995 to present were collected related to the detection/testing of estrogen-, androgen-, and thyroid-active chemicals in the following test systems: cell lines, primary cells, fish/frog embryos, yeast, bacteria, cell free systems, and 'omics technologies. A critical analysis was performed to (1) determine the strengths and limitations of each alternative assay identified and (2) present conclusions regarding chemical specificity, sensitivity, and correlation with in vivo data. A summary of the most promising alternative assays will be presented.

W 2467 THE OECD FISH TESTING FRAMEWORK PROJECT.

L. Touart[1], G. Ankley[2], M. Embry[3], A. Gourmelon[4], T. Iguchi[5], G. Maack[6], P. Matthiessen[7], J. Wheeler[8] and C. Willett[9]. *[1]OCSPP/Office of Science Coordination and Policy, U.S. EPA, Washington, DC, [2]Office of Research and Development, NHEERL, Mid-Continent Ecology Division, U.S. EPA, Duluth, MN, [3]ILSI Health and Environmental Sciences Institute, Washington, DC, [4]Organisation for Economic Cooperation and Development (OECD), Paris, France, [5]National Institute for Basic Biology (NIBB), Okazaki, Japan, [6]Umweltbundesamt (UBA), Dessau, Germany, [7]Consultant, Ulverston, United Kingdom, [8]Syngenta, Bracknell, United Kingdom and [9]PETA, Norfolk, VA.*

A project in the OECD Test Guidelines Programme for considering aspects of a Fish Testing Framework was initiated in mid-2009, with the United States as the lead country. The objectives of the project are to review the regulatory needs and data requirements for fish testing and appraise the currency and comprehensiveness of existing fish OECD Test Guidelines. In addition, the project aims to support animal welfare concerns by identifying unnecessary test methods, minimizing the number of in vivo fish tests and numbers of fish used, and ensuring the optimal use of data derived from in vivo studies. A September 2010 workshop held at Jealott's Hill in the United Kingdom with participation from 50 experts was organized by OECD with the goal of producing a Fish Testing Framework guidance document that provides a detailed discussion of issues, relevant endpoints, and the recommendation for a harmonized testing framework for fish. The workshop participants also reviewed existing and proposed OECD fish-related Test Guidelines and proposed recommendations for deleting, adding, and updating these tests. This presentation will highlight the outcome of the September workshop and discuss the developed framework document.

W 2468 DE-RISKING THE POTENTIAL FOR CARDIOVASCULAR TOXICITY OF TYPE 2 DIABETIC DRUGS: PRECLINICAL AND CLINICAL STRATEGIES.

A. S. Bass[1] and P. K. Hoffmann[2]. *[1]Global Safety Assessment, Merck & Co., Kenilworth, NJ and [2]Preclinical Safety, Novartis, East Hanover, NJ.*

Discovery of effective therapies for the treatment of Type 2 Diabetes (T2D) remains an important and critical unmet medical need for society. As with the development of most new pharmaceuticals, the risk of failure of a compound to achieve marketing authorization remains high. As a result, the pharmaceutical industry has placed a strong commitment in implementing strategies that de-risk the chance of failure when a compound is selected for development. This commitment is reflected in the increasing complexity of safety evaluations and dedication of resources as a compound advances through development. Ultimately, the experiences gained over the course of this time informs whether the de-risking strategy pursued has been effective. As shown in the area of development of T2D drugs, the knowledgebase upon which decisions are made is limited because scientists do not fully understand the relationship of different therapeutics for T2D to unwanted cardiovascular toxicities. However, standard models of cardiovascular toxicity have been established and provide some reassurance of a compound's safety. In those cases, though, where there is evidence of a relationship, investigators still cannot say how well their preclinical models and early clinical phase monitoring predict the observed outcome when a therapy is given over a life-time. Against this backdrop a debate of best practices in cardiovascular safety testing will ensue based on a current understanding of the state of science in the areas of safety pharmacology, toxicology-pathology, and clinical pharmacology. Ultimately, our panel of experts will advocate for integration of information emerging from each of these scientific disciplines that will lead to de-risking the potential adverse cardiovascular outcomes of T2D drugs.

W 2469 ADVERSE CARDIOVASCULAR RISK ASSOCIATED WITH THERAPEUTICS FOR TYPE 2 DIABETES: CHALLENGES TO IDENTIFYING ACCURATE, PREDICTIVE PRECLINICAL MODELS OF TOXICITY.

A. S. Bass. *Global Safety Assessment, Merck & Co., Kenilworth, NJ.*

A large comprehensive outcome trial of the cardiovascular risk posed by drugs for the treatment of Type 2 diabetes is now required by the FDA in support of marketing authorization. The challenges for preclinical scientists is to identify a compound's risk as early as possible, but no later than early clinical development, in an effort to mitigate the high probability of attrition and focus resources on those molecules with the best chance of success. While the adversities of certain pharmaceuticals have been chronicled over the last several years, mechanisms for these adverse events are not necessarily well understood. This uncertainty poses a challenge for the scientist to identifying predictive models of cardiovascular risk. An additional challenge is that in many cases, the investigator is to predict a cardiovascular event that only emerges following years to decades of treatment. The fact is that not all cardiovascular adverse events of drugs for the treatment of T2 diabetes will be amendable to detection in preclinical assays. Accepting these challenges though, the preclinical scientist understands many of the mechanisms of pharmacodynamic and structural cardiovascular toxicity can be accurately modeled using sophisticated methodologies that may also be applied to clinical monitoring for potential untoward cardiovascular events. Thus, the issues related to the safe and expeditious progression of drugs for the treatment of Type 2 diabetes through development have moved the preclinical safety pharmacologist, toxicologist-pathologist and the clinical pharmacologist to focus their efforts on developing integrated strategies that rely on established models and technologies of monitoring for cardiovascular safety of newly emerging molecules. The purpose of this talk is to introduce the challenges of identifying cardiovascular risk in preclinical and clinical study of new molecules for the treatment of Type 2 diabetes and to suggest ways of mitigating that risk, setting the backdrop to the workshop presentations.

W 2470 MODELING CARDIOVASCULAR TOXICITY OF THERAPIES FOR TYPE 2 DIABETES *IN VITRO.*

P. Hoffmann. *Novartis, East Hanover, NJ.* Sponsor: P. Bentley.

Modeling cardiovascular toxicity in vitro provides the highest capacity means of screening molecules in an early stage of discovery and development. Furthermore, there are several in vitro models that emulate and predict the risk of cardiovascular toxicity in human following exposure to a new molecule. However, the lack of an understanding of the mechanisms associated with the cardiovascular toxicity of a life-time of therapy hinders the application of these models to predicting such a result. The solution to this dilemma is to apply those models of cardiovascular risk that have been established and to secondarily attempt to identify mechanisms of toxicity specific to treatments for Type 2 diabetic drugs and to develop models that emulate this condition as a basis for excluding those chemicals with a potential for undesirable properties.

W 2471 CARDIOVASCULAR TOXICITY OF TYPE 2 DIABETES DRUGS, PRECLINICAL *IN VIVO* MODELS AND SPECIALIZED PROTOCOLS FOR IDENTIFYING CARDIOVASCULAR RISK.

P. C. Levesque. *Discovery Toxicology, Bristol-Myers Squibb, Pennington, NJ.* Sponsor: A. Bass.

Patients with diabetes are at increased risk for cardiovascular (CV) disease, which is the predominant cause of death in diabetic patients. Pathogenic mechanisms linking hyperglycemia to microvascular complications like diabetic nephropathy and neuropathy and macrovascular complications like atherosclerosis and coronary artery disease include endothelial dysfunction, dyslipidemia, oxidative stress and impaired vasomotor response. The Endocrinologic and Metabolic Drugs Advisory Committee for the FDA has recently acknowledged that while it remains sufficient for diabetes drug candidates to improve glycemia to be considered to have clinical merit, clinical trials should be designed to rule out an unacceptable increase in CV risk. While FDA stopped short of requiring demonstration of CV benefit, the growing interest within industry to identify new drug targets for Type 2 diabetes with potential CV benefit, such as the incretin-based therapies, highlights concern over demonstration of CV safety. The treatment of Type 2 diabetes is complicated by the physiologic responses to changes in glucose levels and the on- and off-target activities of test agents. Modeling these effects in preclinical assays is likewise complicated, but this can be managed under controlled settings. The application of various preclinical in vivo models to evaluate CV risk of new molecules targeted for the treatment of Type 2 diabetes will be presented. Different strategies for CV risk assessment can be applied in a program specific manner depending on potential for target- or structure-based toxicities, and could include routine safety pharmacology models in normal animals to identify general CV risks, specialized models with a focus on known CV pathologies and risk factors associated with diabetes or toxicology endpoints in chronic efficacy studies. The complexities of disease models, physiologic changes associated with therapeutic effect, and the translation of results from these in vivo investigations to identifying human risk will form the basis for a debate of best practices.

W 2472 PRECLINICAL MODELING OF CARDIOVASCULAR RISK FOR TYPE 2 DIABETES DRUGS: A PATHOLOGIST'S PERSPECTIVE.

B. R. Berridge. *Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC.* Sponsor: A. Bass.

Preclinical modeling of clinical safety risks for drugs intended for long term administration in target patient populations with complicated disease is challenging. Current paradigms for risk identification, albeit broad and multifaceted, are limited and do not address some key sources of adverse outcomes. This presentation will review a typical preclinical safety assessment package with particular emphasis on the components of the package that inform cardiovascular safety. Common findings and their toxicologic relevance will be noted. Also, significant gaps in our traditional paradigms will be explored- particularly in the context of target patient populations with diabetes mellitus and heightened cardiovascular disease risk. Novel opportunities to address these gaps will also be explored and put in the context of traditional approaches. The goal of this presentation is to stimulate interest and discussion in and about innovative approaches to preclinical modeling of risk for therapeutics with greatest potential for long term adverse outcomes.

W 2473 CLINICAL MODELING OF CARDIOVASCULAR TOXICITY OF TYPE 2 DIABETES DRUGS: REGULATORY EXPECTATIONS AND EXPERIENCE.

D. E. Gutstein. *Clinical Research, Merck & Co., Rahway, NJ.* Sponsor: A. Bass.

Study of drugs for potential cardiovascular toxicity in clinical trial patients may be complicated by the underlying disease. This is certainly true in the development of drugs for Type 2 diabetes and has prompted the FDA to issue guidelines specific to cardiovascular testing prior to a products registration. Our understanding of the relationship between treatment and adverse effect is not always well known, further complicating the study of new molecules. As a result, the integration of the series of preclinical assays of efficacy and off-target activity and the demonstration of cardiovascular toxicity identified during the clinical development of a new drug using robust analytical methodology forms a basis for understanding the risk posed by a new drug that allows authorization of marketing approval.

W 2474 MEETING THE CHALLENGES OF RESPIRATORY TOXICOLOGY TESTING -IN SEARCH OF BEST PRACTICES.

J. Wagner[1] and F. Cassee[2]. [1]*Michigan State University, East Lansing, MI and* [2]*RIVM, Bilthoven, Netherlands.*

Inhalation exposure to airborne materials in the home, workplace, and outdoors can produce adverse responses in the airways of both healthy and compromised individuals. Creating comparable exposure/response scenarios in laboratory animals presents significant challenges for the respiratory toxicologist, and over the last decade a number of new approaches have been adopted that complement or replace older practices. However many of these new advances are neither widespread nor widely accepted. Indeed the choices of airway exposure, and types and methods of data collection can provide the researcher with an array of disparate results, thereby creating a challenge for meaningful risk assessment. We will examine the value of popular and perhaps controversial methodological approaches to evaluate toxic airway responses. Beginning with a critical survey of exposure techniques, presentations will expand into the limitations of animal models, the predictive value of acute responses for chronic and functional responses, the special challenges with testing nanomaterials, and the evolution on in vitro approaches. Together the presentations are intended to foster scientific discussions and debate that may unify our thinking on the best practices for respiratory toxicology testing, and in the long term provide research tools to improve human health.

W 2475 DOSING THE RESPIRATORY TRACT.

G. Oberdörster. *Environmental Medicine, University of Rochester, Rochester, NY.*

Animal models of human inhalation exposure employ a number of delivery approaches, including intranasal and intratracheal instillation, aspiration and insufflation methods, in addition to nose-only and whole body inhalation. These techniques have their advantages and disadvantages, and the value of the resulting data is sometimes open to question. Ten years ago the IRSS published a white paper pointing out limitations with regard to intratracheal approaches in toxicity testing. While inhalation is to be viewed as the gold standard for physiological delivery of airborne materials, it requires also the most effort in terms of technical and monetary resources. Thus, because of the ease of delivery the alternative methods have become widespread for administering defined doses to the respiratory tract. Interpretation of results has to be done with great caution, which becomes even more difficult when delivering nanoparticles that are surface modified for intratracheal dosing being in the pristine state for dosing by inhalation. Usefulness and limitations with respect to the relevance of dose and dose rate, for hazard identification and risk assessment of the administered material, for evaluating mechanisms and determining biokinetic pathways need to be considered when selecting a specific dosing method. Models that predict deposition of inhaled particles throughout the respiratory tract can be used to evaluate the relevance of doses administered by the different methods. This presentation will provide a thorough comparison of the strengths and weaknesses of airway exposure methods currently used in inhalation toxicology.

W 2476 CAN ANIMAL INHALATION AND INTRATRACHEAL INSTILLATION (IT) STUDIES BE MADE TO SIMULATE HUMAN EXPOSURE SCENARIOS?

G. E. Hatch. *National Health and Environmental Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC.*

The use of animals (especially rodents) in estimating the toxicity of inhaled agents for humans now has a long history. Some risk assessors are eager to discard animal studies as being irrelevant to humans, while others might over-interpret animal findings. This presentation will explore whether animal inhalation and IT studies can be made comparable to human exposure scenarios as related to dose to the sensitive lung tissues. It will draw from studies using labeled ozone, phosgene and nitrogen dioxide which have attempted to measure dose across species through the use of reaction product (adduct) concentrations and compare adduct with effect measurements. It will show some examples of how linkages can be made between species and exposure scenarios that can eventually predict acute effects in humans based on animal or in vitro data. The dose of inhaled gases and particles to sensitive deep lung tissues is affected by activity level, reflex limitation of breathing, nasopharyngeal removal and biochemical defenses – all of which can show large animal-human differences. Examples will be shown of progress toward understanding the contribution of the above factors across species. Bronchoalveolar lavage (BAL) measurements of dose (adduct), effect (protein leak) and antioxidant content can enable good comparisons between species. Our data show that although allometric

relationships predict that resting rats should breathe almost 3 times as much reactive gas per lung weight as humans, differences in activity, antioxidant concentrations, and reflex limitation of breathing make rat lungs accumulate a low dose of ozone compared to exercising humans or resting Rhesus monkeys. If rats are exposed to higher concentrations of ozone, human-like effects can be achieved. In summary, the measurement of tissue-specific dose and effect markers in BAL can enable dosimetric comparisons between humans and animal species, and can enable animal inhalation exposures to simulate human exposures to reactive gases.

W 2477 SAMPLING THE AIRWAY: IMPROVING THE PREDICTIVE AND TOXICOLOGICAL VALUE OF BRONCHOALVEOLAR LAVAGE.

U. P. Kodavanti. *EPHD/NHEERL/ORD, U.S. EPA, Research Triangle Park, NC.*

Bronchoalveolar lavage (BAL) is a relatively simple technique to obtain biological material in the form of BAL fluid (BALF) from airways of humans and laboratory animals. Numerous predictive biomarkers of pulmonary injury and diseases can be detected in BALF which aid in diagnosis and understanding mechanisms. Cells obtained from BALF can be cultured and/or used for determining transcriptional responses. Because BAL can be done both in animals and humans, it is amenable to animal-human extrapolation. As a terminal procedure in small animals, its value outside the context of pathological or functional change is limited; however, identification of novel biomarkers can provide mechanistic insights. Generally alveolar infiltration of neutrophils, eosinophils and lymphocytes correlates well with the pathological indices in acute toxicity; however, the BALF macrophage increases need to be interpreted in conjunction with other biomarker assessments. The identification of environmental chemicals, insoluble particulate materials and infectious agents in BALF allows one to determine the cause and interpret acute injury to potential chronic outcome. Temporal assessment of antioxidants, cytokines, mucins, and changes in structural lung components can provide insights into disease progression; however, one must take into account the commonalities in the biomarkers between diseases for diagnostic purpose. Assessment of multiple biomarkers together with functional assessment of the lung and pathology often are necessary in interpretation of change. Newer approaches for collection and analysis of epithelial cells in animals may have translational value for biomarkers derived from humans. Airway specific biomarkers when analyzed in BALF and also in plasma/urine provide conclusive diagnostic values. The use of proteomic and transcriptomic approaches in assessment of biomarkers in BALF can accelerate our understanding of complex pulmonary diseases. This presentation will assess the predictive value of old and new emerging BALF endpoints in acute and chronic toxicity studies. (Does not reflect US EPA policy).

W 2478 SCREENING FOR TOXIC AIRWAY RESPONSES: THE COMPARATIVE TOXICOLOGICAL VALUE OF PULMONARY FUNCTION TESTING APPROACHES.

J. Wagner. *Michigan State University, East Lansing, MI.*

Toxicant exposure to the respiratory tract can induce inflammatory and structural changes in airways that may or may not compromise breathing function. Conversely, irritant and neurogenic responses to exposures may result in altered pulmonary function without detectable inflammation or structural changes. As such, measurements of both physiological and structural responses are important to adequately understand the risks and hazards of potential toxicants. The use of whole body plethysmography (WBP) captures respiratory timing parameters, interpretations of which are questioned by physiologists. However this approach minimizes the use of laboratory animals and can be an effective tool for large-scale toxicological studies. By comparison, intubated and ventilated procedures provide more direct airway resistance responses, but are usually terminal procedures (unlike WBP). However these types of data perhaps translate better to clinical measures in humans. The goal of this presentation is to compare noninvasive and invasive approaches to quantify airway functional responses in laboratory animals by using experimental results to illustrate their strengths and weaknesses, with the goal of proposing their most reasonable use in inhalation toxicology

W 2479 SPECIAL CONSIDERATIONS FOR THE DELIVERY OF NANOMATERIALS TO THE RESPIRATORY TRACT: IMPLICATIONS FOR INTERPRETATION OF RESULTS.

A. Elder. *Environmental Medicine, University of Rochester, Rochester, NY.*

Respiratory toxicology studies using nanomaterials present a unique set of challenges, especially with regard to particle preparation and exposure methodologies. The nanotoxicology literature provides several examples where comparable studies have resulted in disparate conclusions. These disparities can be related to factors such as sample handling during preparation for exposure, the presence of dispersants to control particle size, and the means of particle dispersion prior to and during exposure. This presentation will provide a discussion of current approaches and unique problems associated with nanomaterials, with the goal of proposing a reasonable research framework that is aimed at unifying research practices in inhalation toxicology.

W 2480 SCREENING FOR TOXIC AIRWAY RESPONSES: THE TRANSLATIONAL VALUE OF *IN VITRO* APPROACHES.

R. Devlin. *U.S. EPA, Research Triangle Park, NC.*

There are increasingly large numbers of inhaled pollutants whose toxicity needs to be assessed. The cost and time associated with doing these studies in humans or animals, as well as potential ethical issues, make the use of in vitro pulmonary models an attractive alternative. These models can rapidly and cheaply screen many different compounds, and the application of newer molecular approaches can explore mechanisms by which airborne toxicants affect pulmonary cells. In addition, recent advances in cell culturing and exposure systems make these models more "in vivo" like than previous in vitro models. Nevertheless, it remains to be seen how well these newer models reflect in vivo responses. This presentation will focus on the strengths and limitations of using in vitro models as well as provide an update on recent work which has directly compared data from in vitro models with data generated from exposure of humans to air pollutants.

W 2481 POLISHING TODAY'S JOB CANDIDATE IN A TOUGH ECONOMY.

A. K. Farraj. *Environmental Public Health Division, NHEERL, ORD, U.S. EPA, Research Triangle Park, NC.*

An overall stubbornly high unemployment rate coupled with industry downsizing and institutional program cuts has fostered a tepid job market for toxicologists. Fewer jobs mean greater competition for limited openings. Thus the onus is on job candidates to make sure that they are putting their best foot forward during the job-seeking process. To that end, job candidates must not only polish their research credentials, but also their job-seeking skills. Therefore, our panel of experts from divergent fields will discuss the importance of having a well-rounded portfolio. Candidates will come to understand the value of a good publication record, acquiring experience and skills outside of the laboratory, and the necessity for sound communication skills. It has become increasingly important that job seekers be aware of the job application process including writing a resume/CV and interviewing. Our panel will provide some insight and share some insider information relative to the hiring practices of human resource departments. Additionally, there will be a discussion of the common unflattering mistakes and, or pitfalls that candidates can sometimes make and ways to avoid them.

W 2482 PUBLICATION IS THE FOUNDATION OF A COMPETITIVE JOB APPLICATION.

G. B. Corcoran. *Pharmaceutical Sciences, Wayne State University, Detroit, MI.*

Publication is the first and most fundamental criterion for launching a successful employment application. Publication is necessary, although in itself not sufficient, to open doors and earn interviews. Without a competitive publication record, however, candidates who have great oral and written communication abilities, have done their homework to produce an impressive and informed cover letter, and have enjoyed accomplishments and honors which are the building blocks of a noteworthy curriculum vitae can be and are passed over for more well published candidates. This presentation will review some of the strategies for building your publication record, will offer tips and approaches for preparing effective manuscripts for submission, and will review the manuscript acceptance process including how to increase the odds of your manuscript being accepted and published.

W 2483 STAND OUT AMONGST YOUR PEERS: WAYS TO DISTINGUISH YOURSELF BEYOND PUBLICATION RECORD.

I. Jaspers. *University of North Carolina at Chapel Hill, Chapel Hill, NC.*

Considering how competitive the job market is, it is now more important than ever to make sure that you stand out as a candidate. Besides completing a solid research project and publishing your results, there are several ways you as an applicant can

obtain "additional lines on a resume" that distinguishes you from most other applicants. Among these are obtaining certificates in job-related fields, participation in committees, mentoring/teaching, applying and receiving awards, as well as interests outside the lab all of which demonstrate your future employer that you have enthusiasm, leadership skills, are a team-player, and are able and willing to compete. This presentation will discuss potential opportunities available at most Universities, over the internet, and surrounding communities as well as provide examples on how applicants can acquire experiences that make you stand out amongst your peers.

W 2484 COMMUNICATION SKILLS: AN ESSENTIAL ELEMENT TO YOUR PORTFOLIO.

D. L. Costa. *Office of Research & Development, U.S. EPA, Research Triangle Park, NC.*

As scientists we are trained to think logically using innovative tools to ferret out answers to our research questions or test our hypotheses. We are trained in focused science and we have accepted the responsibility of knowing all there is know about that science question or given topic. But it is how we communicate that science by which we as scientists are often judged. Even when we talk with our peers or seek career opportunities, our science is often judged on how well we deliver the science message – which in the end, is a direct reflection of who we are as scientists, colleagues or coworkers. While some of us may be gifted communicators, most of us are introverts and spend our leisure time reading science to get the next great idea and less on literature to hone our use of language. Nor do we spend a lot of time speaking to varied groups who may not be "subject matter experts" but who need to understand, i.e., selling ourselves. Appreciating these facts is the first step in developing communication skills as an essential element of our portfolios. Those skills include organized and focused writing, framing the essence of the message and delivering that message at whatever level of detail is appropriate to the intended audience, and ensuring that it is as succinct and clear as possible. In our oral communications, clarity and focus are critical to selling ourelves to the audience. Cluttering the important data or message with ancillary (though not necessarily unimportant) information, or worse overly dense visual aids can kill a presentation and the desired personal image we want to communicate as a blossoming scientist. This tutorial will focus on the elements (and do's and don'ts) of good communication - written, visual and oral – that the young scientist should incorporate into his/her skill-mix moving ahead in the professional world. These skills are invaluable in getting the position you want, being effective in that position and moving up the professional ladder, and, not to be forgotten, when interfacing with the public who is our ultimate stakeholder.

W 2485 WHAT IS THE EMPLOYER LOOKING FOR? INSIGHT FROM A JOB PLACEMENT PROFESSIONAL.

T. Leyden. *The Leyden Group, Greenwood Village, CO.* Sponsor: A. Farraj.

While it is important to seek appropriate scientific training and strive to round out one's experience in a variety of ways, perhaps two of the most critical elements of one's success in obtaining a job offer are well-written resumes/CVs and effective interviewing. Terry will share his experience in portraying what employers in the chemical, pharmaceutical, consumer product and contract research industries seek from job applicants. Terry will offer useful tips in resume/CV preparation, how to get your resume/CV in the hands of the employer, and the job interview.

W 2486 AN INSIDER PERSPECTIVE: HIRING PROCESSES WITHIN THE BIOTECH/PHARMACEUTICAL INDUSTRY.

R. Ragsdale. *HR, Genentech & Roche, South San Francisco, CA.* Sponsor: A. Farraj.

This presentation will give prospective job seekers an insider look into key hiring & recruiting methods within HR departments. Learn how to best position yourself to be selected in the job application and interview process by informing yourself on key HR practices and what hiring managers look for. Key topics to be discussed include types of positions (e.g., full time, contract, post doctoral fellowship, internship, co-op), qualifications (to include education, certification, and work experience), the application process (focus on who the key players are, where they look for talent, time lines, how to set yourself apart in the informational interviews, on phone screens and during on-site interviews, selection & decision making process, and offers). Lastly, we'll spend a few minutes discussing transitioning from academia to industry; and from CRO to pharmaceutical/biotechnology companies.

PL 2487 TISSUE CONCENTRATIONS AND IMMUNOLOGICAL EFFECTS IN C3H/HEJ MICE, FOLLOWING AN INHALATION EXPOSURE TO TWO PARTICLES SIZE OF BE AND BEAL.

A. Adam-Poupart[1], C. Muller[1], F. Salehi[2], B. Mazer[2], G. Chevalier[3], Y. Cloutier[3], G. Truchon[4], J. Lambert[5] and J. Zayed[1]. [1]*Department of Environmental and Occupational Health, Faculty of Medicine, University of Montreal, Montreal, QC, Canada,* [2]*Meakins-Christie Laboratories, McGill University, Montreal, QC, Canada,* [3]*Department of Biological Sciences, University of Quebec in Montreal, Montreal, QC, Canada,* [4]*Institut de Recherche Robert-Sauvé en Santé et en Sécurité du Travail, Montreal, QC, Canada and* [5]*Department of Social and Preventive medicine, Faculty of Medicine, University of Montreal, Montreal, QC, Canada.*

Beryllium (Be) is a naturally occurring element essential for the energy, medical and electronics industries. Used in solid form, this element doesn't show health hazard for the public. However, workers exposed through inhalation may develop acute sensitization (BeS), chronic beryllium disease (CBD) and lung cancer. Be toxicity is influenced by many factors, such as concentration, chemical form, solubility and particle size. This study investigated the impact of particle sizes (Mass median aerodynamic diameter ranging between 1.5 and 6.5 μm) of two Be chemical forms (Be metal and Be aluminum) on Be-induced toxicity. Two hundred male mice C3H/HeJ equally divided into 5 groups were exposed by inhalation (nose-only) during 3 consecutive weeks, 5 days/week, 6 hr/day. The first group,exposed to HEPA-filtered air, was used as a control,while the four other groups were exposed to approximately 250 μg/m3 of Be: 1.5 and 4.1 μm and BeAl 4.4 and 6.5 μm. The concentrations of Be were measured in lung, liver, kidneys, spleen and blood while the levels of cells expression and cytokines in spleen (CD4+, CD8+, CD19 and IFN-γ flow) and in bronchoalveolar lavage (IL-2, IL-4, IL-12, TNF-α and IFN-γ) were quantified. Histological scores of lung were also classified by the severity of inflammation. Overall, tissues concentrations and cytokine levels were higher and pulmonary inflammation was greater in the group exposed to smaller particles compared to the group exposed to larger particles for Be and BeAl. These results suggested that particle size might be a critical parameter in the Be-induced toxicity.

PL 2488 CIGARETTE SMOKE EXTRACT ENHANCED AMBIENT ULTRAFINE PARTICLE-INDUCED OXIDATIVE STRESS IN ENDOTHELIAL CELLS.

Y. Mo[1], R. Wan[1], S. Chien[2], D. J. Tollerud[1] and Q. Zhang[1]. [1]*Environmental and Occupational Health Sciences, University of Louisville, Louisville, KY and* [2]*Surgery, University of Louisville, Louisville, KY.*

A number of epidemiological studies have shown that cigarette smoke induces cardiovascular diseases. However, we have identified no studies that address the potential combined effects of UFPs and cigarette smoke on vascular endothelial cells. We hypothesize that co-exposure to UFPs with cigarette smoke extract (CSE) may cause combined effects on activation of endothelial cells and dysfunction of endothelium by oxidative stress through activation of NADPH oxidase. We determined the combined effects of UFPs with or without CSE on mouse pulmonary microvascular endothelial cells (MPMVEC) obtained from wild-type mice and gp91phox knock-out mice, respectively (Gp91phox is one of the key components of NADPH oxidase, which is located in the cell membrane). Our results showed that exposure of MPMVEC from wide-type mice to UFPs, CSE or UFPs with CSE, at a non-toxic dose, induced reactive oxygen species (ROS) generation, increased phosphorylation of p38 and Erk1/2, and up-regulation of early growth response -1 (Egr-1) and IL-6 expression. Furthermore, exposure to UFPs with CSE caused significantly more ROS generation in MPMVEC from wide-type mice than those of exposure to UFPs or CSE alone. Our results also showed that there were combined effects, including increased phosphorylation of p38 and Erk1/2, and up-regulation of Egr-1 and IL-6 expression when MPMVEC from wide-type mice were exposed to UFPs with CSE. However, exposure of MPMVEC from gp91phox knock-out mice did not induce the above effects. Our results suggest that UFPs, CSE, and UFPs with CSE caused the activation of NADPH oxidase. This causes ROS generation that leads to activation of MAPKs through induced phosphorylation of p38 and ERK1/2 MAPKs, and upregulation of Egr-1 that may further result in endothelial dysfunction through production of cytokines such as IL-6. Our results suggest that co-exposure to UFPs and cigarette smoke causes synergistic injury to endothelial cells. Grant Supports: KSEF-1686-RED-11, T32-ES011564.

PL 2489 COMPARATIVE TOXICITY OF BIODIESEL EXHAUST AND PETROLEUM DIESEL EXHAUST PARTICULATE MATTER USING WKY RAT ALVEOLAR MACHROPHAGES.

L. Bhavaraju[2], J. Shannahan[2], A. Williams[3], R. McCormick[3], J. McGee[1], U. Kodavanti[1] and M. Madden[1]. *[1]ORD, NHEERL, EPHD, U.S. EPA, Chapel Hill, NC, [2]Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and [3]Fuels Performance Group, National Renewable Energy Lab., Golden, CO.*

Exposure to fine ambient particulate matter <2.5um (PM2.5) can induce airway inflammation, cardiopulmonary morbidity and mortality. Combustion of petroleum diesel and biodiesel contributes to PM2.5. Possible toxicity caused by inhalation of biodiesel emission particles (BioDEP) have not been well studied compared to petroleum diesel emission (PetDEP). Biodiesel is an alternative fuel source for use in diesel engines that is commonly used as a 20% blend (B20) containing petroleum diesel in the US. In order to study the effects of BioDEP compared to PetDEP, we collected WKY alveolar macrophages (AM) via lavage and exposed the cells in vitro. We observed dose dependent cytotoxicty when exposed to B20 and DEP for 24 hours assessed by LDH activity release. Using non-cytotoxic exposures, AM exposed to concentrations as low as 1μg/mL of B20, but not PetDEP, produced a statistically significant increase in the amount of the immune mediator Prostaglandin E2 (PGE2) when compared to untreated AM's after 24 hours of exposure. Due to significant binding of released PGE2 to PM, cellular mRNAs were examined. 100μg/mL PetDEP significantly upregulated COX2 expression (involved in PGE2 production) relative to control AM. AM were incubated with endotoxin post PetDEP and B20 exposure, and the AM's showed a decreased production of PGE2, suggesting the macrophages have a diminished protective response to bacteria after exposure to PM. These data suggest that both diesel combustion particles can alter AM lipid metabolism and these responses may play a role in the health effects from exposure to ultrafine PM. [This is an abstract of a proposed presentation and may not necessarily reflect official US EPA Policy.]

PL 2490 GSTP1 POLYMORPHISM DETERMINES SUSCEPTIBILITY TO ACUTELY DECREASED PROTEASOME ACTIVITY FOLLOWING HUMAN INHALATION OF FRESH AEROSOLS.

H. M. Kipen[1], S. Gandhi[1], D. Rich[2], P. Ohman[2], R. Laumbach[1], Z. Fan[1], L. Chen[3], J. Zhang[2] and K. Madura[3]. *[1]Department of Environmental & Occupational Medicine, UMDNJ-Robert Wood Johnson Med. School, Piscataway, NJ, [2]UMDNJ-School of Public Health, Piscataway, NJ and [3]Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.*

Background. Epidemiologic studies document that cardiovascular events occur acutely following air pollution increases, yet underlying mechanisms require clarification. The proteasome (UPP), a protein degradation pathway, plays a role in regulating several mechanisms that participate in acute air pollution responses: inflammation, nitric oxide synthesis, and oxidative stress responses. The proteasome can also be inhibited by oxidative stress, suggesting that air pollution may decrease proteasomal activity. We hypothesized that acute exposure to air pollution affects proteasomal activity, particularly in those with genetic susceptibility to oxidant stress. Methods. 38 healthy subjects volunteered for a 2 hour, double-blind, randomized cross-over protocol to breathe diluted diesel exhaust (DE), a secondary organic aerosol (SOA), or clean air (CA). Subjects were stratified based on a GSTP1 polymorphism. UPP activity was examined in peripheral blood cells. Results Compared with CA, WBC showed decreased UPP activity for both DE, -1070.9 (95% CI = -2635.6 to 493.7) and SOA, -1928.5 (-3665.1 to -191.9), and for DE and SOA combined, -1418.1(-2811.3, -24.8). RBC's showed similar although borderline significant effects. Change in proteasomal activity demonstrated a significant interaction between GSTP1 status and SOA exposure, -5145.2 (-8817.3, -1473.0) ($p<0.006$). Conclusions: In two peripheral blood cell types, two different air pollutants reduced the activity of a key regulatory enzyme system. Moreover, individuals homozygous for a variant allele (ILE/ILE) of an antioxidant gene showed a greater response, suggesting the importance of oxidative stress in responses to air pollution exposure.

PL 2491 EXPOSURE DURING ALLERGIC SENSITIZATION TO URBAN SUMMER AND WINTER SAN JOAQUIN VALLEY COMBINED FINE/ULTRAFINE CONCENTRATED AMBIENT PARTICLES IN A MOUSE MODEL OF ALLERGIC AIRWAY INFLAMMATION.

C. Carosino[1, 2], L. E. Plummer[1, 2], M. J. Kleeman[3] and K. E. Pinkerton[2]. *[1]Pharmacology and Toxicology, University of California Davis, Davis, CA, [2]Center For Health and the Environment, University of California Davis, Davis, CA and [3]Civil and Environmental Engineering, University of California Davis, Davis, CA.*

Rationale: Epidemiological data has suggested correlations between particulate matter exposure and increased asthma symptoms and hospitalizations. The San Joaquin Valley (SJV) of California has some of the highest particulate matter (PM) pollution in the country and asthma symptom prevalence is among the highest in the state. We wished to test the seasonal effects of SJV PM on allergen induced airway inflammation with in-vivo exposures. Objectives: To evaluate if exposure to seasonal SJV ambient PM during allergic sensitization elicits differential increases in allergic airway inflammation. Methods: BALB/c mice were exposed to six days concentrated ambient particulates (CAPs) or filtered air (FA) for six hr/day at an urban in the SJV. Winter exposure concentration was 280μg/m3 and summer exposure was 132μg/m3. Mice were sensitized with 10μg of ovalbumin in 50μl PBS via intranasal aspiration immediately following exposures on days 2, 4, and 6. Mice were then challenged with 1% aerosolized ovalbumin for one hour on days 11, 12, and 13. On day 14, the animals were sacrificed and tissues collected for analysis. Results: Bronchoalveolar lavage exhibited cellular profiles indicative of an allergic response; however summer particles exhibited the greatest differential response despite a lower exposure concentration than winter exposures. Trends were also exhibited in epithelial mucosubstances, antibody levels and lung tissue cytokines supporting an increased Th2 response in summer CAPs exposed animals. Results: Exposure during sensitization to summer urban SJV CAPs as compared to FA sensitized animals resulted in increased allergic airway inflammation. Summer urban CAPs may be more potent in eliciting an exacerbated allergic response in comparison to urban winter CAPs. Funded:CARB/EPRI,USEPA,NIEHS

PL 2492 SECONDHAND TOBACCO SMOKE, ANTIOXIDANT ENZYMES, AND AIRWAY DEVELOPMENT IN MALE AND FEMALE MICE.

L. S. Van Winkle, J. Chan, P. Edwards, V. Rosauro, K. Sutherland and A. Buckpitt. *University of California Davis, Davis, CA.*

Lung disease is the number one killer of children under the age of one. Little is known about the influence of early life second hand tobacco smoke (SHS) exposure on the expression of lung antioxidant systems and cells of the deep lung. We used male and female mice to investigate the effect of postnatal SHS on the early pattern of development of lung antioxidant enzymes. Our previous studies have found differences in these enzyme systems in adult animals exposed since birth. In this study we characterized the response earlier, during postnatal development, at 4 weeks of age. Two groups of mice were exposed to 1 mg/m3 SHS 5d per wk from birth and were sampled either 1) on the same day of exposure (SHS0) or 2) after 2 days of recovery in filtered air (SHS3). We then compared the responses in the SHS exposed mice to an age-matched filtered air (FA) control group. We found that SHS exposure in the postnatal mouse decreases gene expression of the airway epithelial Clara cell marker protein (CCSP) more than 5 fold. This was accompanied by decreased expression of GSTpi and peroxiredoxin 6. Using HPLC methods, we characterized the levels of glutathione (GSH) in microdissected airways and found that male mice had significantly more GSH than female mice of the same age but that SHS exposure affected both sexes similarly; decreasing GSH 4 fold in the SHS0 group. Reduced GSH persisted during the FA recovery period in the SHS3 male and female mice. To test whether reduced GSH would result in increased susceptibility to an oxidant stress, the SHS3 exposed mice were injected with 100mg/kg of naphthalene (NA) i.p. Airway epithelial cytotoxicity due to NA was not increased by SHS exposure suggesting other protective mechanisms, possibly including decreased activation of NA, took place. These studies indicate that SHS exposure changes the expression pattern of key protective antioxidants and related enzyme systmes in the airways of the very young and decreases airway epithelial cellular differentiation. Support: ES06700, ES04311 and FAMRI.

PL 2493 ATMOSPHERIC AEROSOLS FORMED FROM BIOGENIC AND ANTHROPOGENIC PRECURSOR REACTIONS AND COAL COMBUSTION EMISSIONS SHOW MILD VASCULAR TOXICITY COMPARED WITH MOTOR VEHICLE EXHAUST.

J. McDonald[1], M. Doyle-Eisele[1], A. Lund[1], M. Campen[3], E. Knipping[2] and A. Rohr[2]. *[1]Lovelace, Albuqueruque, NM, [2]Electric Power Research Institute, Palo Alto, CA and [3]University of New Mexico, Albuquerque, NM.*

Comparative biological response to inhalation of atmospheres of secondary organic aerosol (SOA), motor vehicle exhaust (MVe), and simulated downwind coal atmospheres (SDCA) was evaluated. SOA was derived from the oxidation of α-pinene (biogenic) and toluene (anthropogenic). The reaction conditions included the addition of nitrogen oxides and with/without sulfur dioxide. The addition of SO2 resulted in the additional presence of organosulfate compounds in the α-pinene (biogenic) but not the toluene atmosphere. MVe was created through a combination of gasoline + diesel exhaust, mixed in proportions to achieve the desired particulate concentration (1:5 proportion of gasoline:diesel particulate matter). SDCA was created through combustion of coal and subsequent addition of addition sulfate and inorganic gases that were projected to be present in air downwind from coal combustion facilities. All exposures were conducted at particle concentrations of 300 μg m-3. Apolipoprotein E knockout (ApoE-/-) mice were exposed for 6 h/d x 7 days. Pulmonary inflammation was assayed by analysis of cell counts/differentials in lavage. Aortas were assayed for transcriptional alterations of endothelin-1 (ET-1), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase-2 (TIMP-2), and heme oxygenase-1 (HO-1). Pulmonary inflammation was absent in all atmospheres. Transcriptional alterations in the aorta were highest in the MVe atmosphere. Only mild responses were observed in SOA, with comparable responses from α-pinene and toluene precursors. Minimal to no vascular response was observed from the SDCA. Research supported by the Electric Power Research Institute.

PL 2494 UNIQUE ASSOCIATIONS OF PM2.5 –INDUCED CHANGES IN HEART RATE VARIABILITY IN SPONTANEOUSLY HYPERTENSIVE RATS WITH WIND DIRECTION AND SPECIFIC SOURCES IN STEUBENVILLE, OH.

J. G. Wagner[1], A. Kamal[2], M. Morishita[2], B. Mukherjee[2], G. J. Keeler[2], J. R. Harkema[1] and A. C. Rohr[3]. *[1]Michigan State University, East Lansing, MI, [2]University of Michigan, Ann Arbor, MI and [3]Electrical Power Research Institute, Palo Alto, CA.*

Short-term increases in fine particulate matter (PM2.5) are associated with acute cardiovascular morbidity and mortality, including stroke and myocardial infarction. The specific components in PM2.5 responsible for these adverse responses are unknown. We exposed spontaneously hypertensive (SH) rats to concentrated air particulates (CAPs) in Steubenville, OH, and measured changes in heart rate (HR) and variability (HRV) as physiological markers of cardiovascular stress. Eight male SH rats were exposed to either Filtered Air (FA) or fine CAPs, 7:30AM- 3:30PM for 13 consecutive days and ECG recordings were used to derive HR and HRV indices of SDNN and rMSSD. High-resolution, 30-minute data streams of HRV and CAPs metrics were analyzed with a mixed models design, and positive matrix factorization (PMF) was applied to 30-minute trace element data to identify pollution sources. CAPs exposure (mean 719 μg/m3), induced bi-directional changes in HR and HRV that were associated with specific trace elements and with the predominant wind directions at our exposure sites, from NE or SW directions. Elevated HR was observed during SW winds and associated with Mo, La, Ce, PM mass, NOx and V. Decreased HR was seen when winds were from the NE, and were associated with S, Se, Rb, Pb, Mn and Zn. Source factors linked to HR changes were metal processing with SW winds, and incineration, lead, and steel and iron processing NE winds. CAPs-exposed rats had significant decreases in SDNN regardless of wind direction, and that was associated with the incinerator factor with NE winds and with metals processing from the SW. Modest changes were detected RMSSD only from the SW, with association with Mo. V and SO2, but without significant associations with a source factor. These data demonstrate unique wind- and source-specific dependence of urban PM2.5 exposure on acute cardiovascular and autonomic responses.

PL 2495 INHALATION OF ULTRAFINE PARTICULATE MATTER INDUCES INFLAMMATORY GENE EXPRESSION IN STRIATUM THROUGH NRF-2 AND NF-κB PATHWAYS IN RATS EXPOSED IN MEXICO CITY.

R. Guerra-Garcia[1], E. Vera-Aguilar[2], G. Gookin[3], M. Uribe-Ramirez[1], A. Campbell[4], J. Camacho[2], M. Kleinman[3] and A. De Vizcaya-Ruiz[1]. *[1]Department of Toxicology, Cinvestav, Mexico City, Mexico, [2]Department Pharmacology, Cinvestav, Mexico City, Mexico, [3]Division of Occupational and Environmental Medicine, Department of Medicine, University of Irvine, Irvine, CA and [4]Department of Pharmacological Sciences, Western University of Health Sciences, Pomona, CA.*

Air pollution particulate matter (PM) consist of transition metals and organic compounds (PAH), which can reach the brain and aggravate events linked to the pathology of brain stroke, Alzheimer's, and Parkinson's diseases. Brain inflammation in apolipoprotein E knockout (Apo $E^{-/-}$) and ovalbumin sensitized mice after exposure to different PM fractions derived from a heavily polluted site in California, USA, while loss of striatal neurons was observed in PM-exposed Apo $E^{-/-}$ mice in a study in New York (NYC). To further investigate the toxicity of PM on central nervous system, Sprague-Dawley male rats were exposed to filtered air or coarse, fine (fine+ultrafine) or ultrafine concentrated PM from Mexico City for 8 weeks, using a particle concentrator system during the warm-dry season of 2009. Olfactory bulb, frontal cortex, striatum and hippocampus, structures linked with neurodegenerative diseases, were harvested after exposure to assess gene expression of proinflammatory cytokines: IL-1β and TNFα using real-time PCR. Activation of nuclear transcription factors NF-κB and Nrf-2, related with inflammatory and antioxidant responses, respectively, was measured by gel shift mobility assay. IL-1β and TNFα increased 2 and 3-fold versus filtered air, respectively, and activation of NfκB and Nrf-2 was observed in striatum of the animals exposed to ultrafine PM. Thus the exposure to ultrafine PM enhances oxidative stress in the brain, which possibly leads to an inflammatory response. These molecular processes might help to explain the linkage between higher incidences of neurodegenerative diseases and exposures to elevated levels of ambient ultrafine PM. (Financement: CONACyT #57752 and UCLA-Fogarty Program).

PL 2496 NRF2 IN TOXICOLOGY: FROM MONOFUNCTIONAL INDUCERS TO THE NEXT GENERATION OF THERAPY.

C. D. Klaassen. *Pharmacology, University Kansas Medical Center, Kansas City, KS.*

In the late 1980s, Paul Talalay pioneered the idea that some inducers had little effect on phase-I enzymes but induced phase-II enzymes. In retrospect, this concept of monofunctional inducers explained many confounding results in toxicology, such as the protective effect of butylated hydroxyanisole against acetaminophen hepatotoxicity and the observation that oltipraz diminished aflatoxin-induced carcinogenicity. In 1990, Cecil Pickett's group reported that induction of glutathione S-transferases (GSTs) was dependent on an antioxidant response element (ARE) found in genes, and in 1997 Yamamoto and colleagues discovered that Nrf2 was the transcription factor necessary for the induction. Thus, many monofunctional inducers are now referred to as Nrf2 activators, which induce genes containing AREs. Our work on Nrf2 began in the late 1980s when Jie Liu came to my laboratory from China with several compounds extracted from Chinese herbal medicines. He tested 16 of these chemicals for hepatoprotective effects and determined one chemical, oleanolic acid, was the most effective. In fact, oleanolic acid protected against nine different hepatotoxicants with seemingly different pathways of producing toxicity. Michael Sporn and his group synthesized many derivatives of oleanolic acid, and eventually 2-cyano-3,12-dioxooleana-1,9-dien-28-imidazolide (CDDO-Im) emerged as the most potent derivative. We now know that Nrf2 activators increase not only the GSTs and other phase-II enzymes, but also glutathione synthesis enzymes, heme oxygenase-1, NAD(P)H:quinone oxidoreductase 1, microsomal epoxide hydrolase, and various enzymes that detoxify reactive oxygen species, all of which cumulatively produce robust anti-oxidant and anti-inflammatory effects. Thus, it is not surprising that activators of the Nrf2 pathway, like oleanolic acid, can protect against the toxicity of many chemicals. It is interesting to note that another derivative of oleanolic acid (CDDO-Me, bardoxolone methyl) is in Phase 2 clinical trials as an activator of the Nrf2 pathway for the treatment of chronic kidney disease in diabetic patients. (NIH DK-081461)

PL 2497 TRANSCRIPTIONAL REGULATION OF THE HUMAN FERRITIN GENE THROUGH AN ANTI-OXIDANT RESPONSIVE ELEMENT BY CORE HISTONE MODIFICATIONS.

B. Huang, K. Iwasaki and Y. Tsuji. *Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.* Sponsor: J. Ninomiya-Tsuji.

Oxidative stress is implicated in various diseases such as cancer and neurodegeneration; therefore, gaining insight into the molecular mechanism through which antioxidant detoxification genes are regulated is important to understand the pathogenesis and prevention of oxidative stress-related diseases. Ferritin, the major iron storage protein that sequesters intracellular excess iron to prevent production of reactive oxygen species, is transcriptionally regulated via an ARE (anti-oxidant responsive element) under oxidative stress. We recently observed that Nrf2 and ATF1 bind to the ferritin ARE. Single knockdown of ATF1 or Nrf2 showed only partial effect, whereas ATF1 and Nrf2 double-knockdown in K562 human erythroleukemic cells showed a complete inhibition of ferritin mRNA induction following hemin treatment. Hemin-mediated transcriptional activation of the ferritin gene was associated with p38 MAPK phosphorylation along with ATF1 phosphorylation at Ser-63, in which SB203580, a p38 inhibitor, inhibited all these events. Concomitantly, both recruitment of protein arginine methyltransferase 1 (PRMT1) and its target methylation of histone H4 Arg-3 around the ferritin ARE were increased after hemin treatment. PRMT1 expression activated ARE-dependent ferritin transcription, and AMI-1, a PRMT inhibitor, inhibited hemin-mediated ferritin transcription. These results suggest that hemin-mediated ferritin transcription through the ARE is regulated by p38-mediated ATF1 Ser-63 phosphorylation along with PRMT1-mediated histone H4 Arg-3 methylation. Furthermore, ChIP assays demonstrated that acetylation of histone H3 Lys-9 and -14, and recruitment of histone acetyltransferases p300 and CBP around the ferritin ARE were induced following hemin treatment. We are currently investigating whether phosphorylation of ATF1 at Ser-63 and recruitment of PRMT1 orchestrate ferritin ARE activation by recruiting chromatin remodeling proteins such as p300 and CBP and subsequent histone acetylation.

PL 2498 ACTIVATION OF NRF2 BY INTRINSIC AND EXTRINSIC LIGANDS.

A. Chia, R. Megherbi, I. Copple, N. R. Kitteringham, L. E. Randle, C. E. Goldring and K. Park. *Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, Merseyside, United Kingdom.* Sponsor: D. Mendrick.

Nrf2 is a key activator of cytoprotective genes in mammalian cells. Cysteine residues in the inhibitor of Nrf2, Keap1, are targets for electrophilic and oxidative modifications, which may result in uncoupling of Keap1 to Nrf2 resulting in Nrf2 stabilization. The work of a number of groups, including our own, has shown that patterns of cysteine adduction in Keap1 in vitro and in living cells are electrophile-dependent. The aim of the present work was to assess the relevance of covalent modification of cysteines in Keap1 in the activation of Nrf2-dependent genes through the use of a model compound, DNCB, which targets only one cysteine in Keap1 expressed in cells, i.e. C257. LC-MS/MS analysis was performed on Keap1 intracellularly modified by DNCB and its analogues (dinitrohalobenzenes), which retain similar chemistry to DNCB, deplete glutathione with similar potencies, but differ in their overall reactivity with intracellular protein targets. We detected modification of intracellular Keap1 by all of these chemicals only at C257, and found that this remarkable cysteine residue selectivity occurs despite the fact that all of the cysteines of Keap1 are potentially available for modification by other sulfhydryl-reactive chemical probes. Pre-treatment of Keap1-expressing cells with buthionine sulfoximine (an inhibitor of glutathione synthesis), resulted in a reduced ability of DNCB to adduct C257. Nevertheless, mutation of this cysteine did not affect the ability of DNCB to induce Nrf2 accumulation and Nrf2-dependent gene transcription. The hypothesis that reversible and/or irreversible oxidation of other cysteine residues in Keap1 may provide a signal for activation of the Nrf2 pathway is currently being pursued. In summary, covalent binding to specific reactive cysteine residues in Keap1 may not necessarily be the sole trigger for activation of Nrf2-dependent cell defence, rather that other forms of modification of Keap1, and possibly other proteins in this pathway, may also serve as sensors, to enable the system to be chemically highly versatile.

PL 2499 KEAP1-KNOCKDOWN AND HEPATOCYTE-SPECIFIC SIRT1 DELETION CONFER RESISTANCE TO FASTING-INDUCED DOWN-REGULATION OF NRF2-TARGET GENE EXPRESSION.

S. Kulkarni[1], J. Xu[1], W. Wei[1], X. Li[2], M. Yamamoto[3] and A. Slitt[1]. [1]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI,* [2]*National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC and* [3]*Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan.*

Food deprivation (fasting) causes a coordinated whole body response to enhance gluconeogenesis. Fasting increases serum glucagon levels; this activates the protein kinase A (PKA)/cAMP signal transduction pathway and enhances liver glucose production. Fasting and caloric restriction also increase Sirtuin 1 (Sirt1) activity, which also enhances gluconeogenesis. Both pathways enhance the expression and activity of the transcriptional co-activator Pgc-1α. Nuclear factor-E2 related factor 2 (Nrf2, Nfe2l2) is a transcription factor, well known to be responsive to cellular oxidative stress, but may be also sensitive to cellular nutrient status. First, C57Bl/6 (lean) and ob/ob (obese) mice were food withheld (fasted) for 30 hours and Nrf2 target gene (e.g. Nqo-1, gclc, gclm, gsta-1, Mrp2) expression was quantified. Fasting for 30 hours decreased Nrf2 target gene expression in liver by 40-75% compared to fed C57BL/6 and ob/ob mice. Second, increased constitutive expression of Nrf2 diminished the fasting effects on Nrf2 target genes (lower fold decrease), as observed in Keap1-KD fasted mice compared to C57Bl/6 fasted controls. Third, Liver-specific deletion of Sirt1 (Sirt1LKO), a deacetylase known to be activated upon nutrient deprivation, also diminished the fold decrease in expression of Nrf2 target genes upon fasting. In general, the obese, Keap1-KD, and Sirt1LKO mice were resistant to fasting-induced changes in Nrf2 target gene expression, as observed by the attenuated expression of Pgc-1α in these livers. The above data directs towards a novel role for nutrient status affecting Nrf2 activity, the antioxidant response, and potential novel interplay between Sirt1 and the antioxidant response (NIH 3R01ES016042).

PL 2500 A BIOCHEMICAL AND PROTEOMIC ANALYSIS OF THE EFFECT OF NRF2 GENE DELETION ON ACUTE ACETAMINOPHEN-INDUCED HEPATOTOXICITY.

C. E. Goldring, L. E. Randle, R. L. Sison, N. R. Kitteringham, D. Denk, R. E. Jenkins, J. Walsh, B. Lane, A. Kipar and K. Park. *Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, Merseyside, United Kingdom.* Sponsor: D. Mendrick.

The Keap1-Nrf2-ARE signalling pathway has emerged as a key protective mechanism in the response to chemical stress. To date, a number of studies have investigated Nrf2-dependent changes at the RNA level, however these may not necessarily be translated into changes at the protein level and function. We have now sought to determine the role of Nrf2 in the early mechanisms of adaptation to acute DILI through a shotgun proteomics approach and targeted metabolite analysis, in a murine model of acetaminophen (APAP)-induced hepatotoxicity. Clinical chemistry and histopathology confirmed the increased sensitivity of Nrf2-null mice towards APAP-induced liver damage. Metabolomic analysis of the glutathione system indicated adaptation to Nrf2 deletion through an enhancement of cellular defence through conservation and/or increase of intermediates in this pathway, in an apparent attempt to maintain redox homeostasis. Proteomic analysis revealed Nrf2-mediated efforts by the liver to maintain energy production. Following acute APAP-mediated injury, it appears that an early Nrf2-mediated attempt is made to source alternative substrates for the citric acid cycle by upregulating enzymes within the hepatoproteome. Liver carboxylesterase 31 and propionyl-coA carboxylase were identified as novel Nrf2-dependent, APAP-responsive proteins. Several other APAP-sensitive proteins were also identified, and these may warrant further investigation into their suitability as biomarkers of APAP intoxication. In summary, we have demonstrated novel APAP-mediated, Nrf2-dependent changes in the liver proteome and metabolome that provides new areas of study for delineating the molecular events that are implicated in this important form of liver toxicity.

PL 2501 PROFILING ENVIRONMENTAL CHEMICALS IN THE ANTIOXIDANT RESPONSE ELEMENT PATHWAY USING QUANTITATIVE HIGH-THROUGHPUT SCREENING (QHTS).

S. J. Shukla[1], R. Huang[1], S. O. Simmons[2], R. R. Tice[3], K. L. Witt[3] and M. Xia[1]. [1]*NIH Chemical Genomics Center, Rockville, MD,* [2]*U.S. EPA, Research Triangle Park, NC and* [3]*National Toxicology Program, Research Triangle Park, NC.*

The antioxidant response element (ARE) signaling pathway plays an important role in the amelioration of oxidative stress, which can contribute to a number of diseases, including cancer. We screened 1408 NTP-provided substances in 1536-well

qHTS format at concentrations ranging from 0.59 nM to 92 μM for their ability to induce this pathway in ARE-bla HepG2 cells transfected with a beta-lactamase reporter gene under the control of ARE and in HepG2 cells transfected with a luciferase reporter measuring Nrf2 specific ARE activation. Raw data were normalized relative to beta-napththoflavone (100%) and DMSO (basal, 0%). Concentration response curves were generated for each substance, with EC50 values and efficacy calculated for substances classified as positive in either assay. We identified 364 and 44 compounds that stimulated ARE in the bla and luciferase assays, respectively, with 34 of these compounds active in both assays. Based on either a positive response in both assays or a structure-activity relationship to an active compound, we re-tested 63 compounds in the two ARE assays and also for their ability to deplete glutathione, as a downstream assay. Fifty-six out of 63 compounds were confirmed for ARE activity and several (e.g., hydroquinone) additionally depleted glutathione levels. Our results indicate the robustness of these two different approaches for assessing the ability of much larger compound libraries to induce ARE. Supported by NIEHS Interagency Agreement Y2-ES-7020-01.

PL 2502 NUCLEAR FACTOR E2-RELATED FACTOR 1 IS INVOLVED IN ARSENIC-INDUCED ANTIOXIDANT RESPONSE IN HUMAN KERATINOCYTES.

R. Zhao[1], Y. Hou[1], P. Xue[1], C. G. Woods[1], J. Fu[1], M. P. Waalkes[2], M. E. Andersen[1] and J. Pi[1]. [1]*The Hamner Institutes, Reaserach Triangle Park, NC and* [2]*NTP, NIEHS, NIH, Research Triangle Park, NC .*

Human exposure to inorganic arsenic, a potent oxidative stressor, causes various dermal disorders, including hyperkeratosis and skin cancer. Nuclear factor E2-related factor 1 (NRF1) plays a critical role in regulating the expression of many antioxidant response element (ARE)-dependent genes. The current study investigates the role of NRF1 in arsenic-induced antioxidant response and cytotoxicity in cultured human keratinocytes. In HaCaT cells, inorganic arsenite (iAs3+) enhanced the protein accumulation of long isoforms (120-140 kDa) of NRF1 in a dose- and time-dependent fashion. The long isoforms of NRF1 induced by iAs3+ were mainly accumulated in nucleus of HaCaT cells. Selective deficiency of NRF1 by lentiviral shRNAs in HaCaT cells (NRF1-KD) led to decreased expression of γ-glutamatecysteine ligase catalytic subunit (GCLC) and regulatory subunit (GCLM) and a reduced level of intracellular glutathione. In response to acute iAs3+ exposure, induction of some ARE-dependent genes, including NAD(P)H: quinone oxidoreductase 1 (NQO1), GCLC and GCLM, was significantly attenuated in NRF1-KD cells. However, the iAs3–induced expression of heme oxygenase 1 (HMOX-1) was unaltered by silencing of NRF1, suggesting HMOX-1 is not regulated by NRF1. In addition, lack of NRF1 in HaCaT cells did not disturb iAs3+-induced NRF2 accumulation, but noticeably decreased KEAP1 protein levels under basal and iAs3+-exposed conditions, suggesting a potential interaction between NRF1 and KEAP1. Consistent with the critical role of NRF1 in the transcriptional regulation of some ARE-bearing genes, knockdown of NRF1 significantly increased iAs3+-induced cytotoxicity and apoptosis. The current study, for the first time, demonstrates that long isoforms of NRF1 contribute to arsenic-induced antioxidant response in human keratinocytes and protect the cells from acute arsenic cytotoxicity.

PL 2503 BENEFICIAL ROLE OF NRF2 IN REGULATING NADPH GENERATION AND CONSUMPTION.

K. C. Wu, J. Y. Cui and C. D. Klaassen. *Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS.*

Nrf2 (nuclear factor E2 p45-related factor 2) is a central regulator that promotes the transcription of cytoprotective genes in response to oxidative and electrophilic stresses. Most functions of Nrf2 were identified by studying biological models with Nrf2 deficiency, however, little is known about the effects of graded Nrf2 activation. In the present study, hepatic phenotypes as well as genomic gene expression profiles by microarray analysis were characterized with a 'gene dose-response' model in livers of Nrf2-null mice, wild-type mice, Keap1-knockdown (Keap1-KD) mice with enhanced Nrf2 activation, and Keap1-hepatocyte knockout (Keap1-HKO) mice with maximum hepatic Nrf2 activation. Hepatic nuclear Nrf2 protein, glutathione concentrations, and known Nrf2 target genes were increased dose dependently. In total, 115 genes were identified to be constitutively induced and 80 genes suppressed with graded Nrf2 activation. Specifically, mRNA of genes encoding enzymes in the pentose phosphate pathway as well as malic enzyme were low with Nrf2 deficiency and high with Nrf2 activation, indicating that Nrf2 is important for NADPH production. NADPH is the major reducing power in the body to scavenge oxidative stress including regenerating glutathione and thioredoxin, and is also used for anabolic pathways including lipid synthesis. HPLC-UV analysis confirmed that hepatic NADPH concentration was lowest in Nrf2-null mice and highest in Keap1-HKO mice. In addition, genes involved in fatty acid synthesis and desaturation were down-regulated with graded Nrf2 activation. In conclusion, the present study suggests that Nrf2 fights against environmental insults to benefit cell survival by promoting the generation of NADPH, and by pushing its consumption into the direction of scavenging oxidative stress rather than fatty acid synthesis and desaturation. (Supported by NIH grants ES-09649, ES-09716, ES-07079, DK081461, and RR-021940)

R 2504 ASSESSMENT OF NANOPARTICLE EXPOSURE IN OCCUPATIONAL SETTINGS AND IN INHALATION TOXICOLOGY STUDIES: IS THERE A BEST DOSEMETRIC TO USE?

D. B. Warheit[1] and G. Oberdorster[2]. [1]*DuPont Haskell Global Centers, Wilmington, DE and* [2]*University of Rochester, Rochester, NY.*

Nanotechnology involves the design and manipulation of materials at the nanoscale size range. The capacity for assessing hazards or quantifying exposures to engineered nanomaterials for workers or consumers is limited, and may require new or different methodologies to provide information for effective risk assessment and risk management. Exposure assessments are important components for determining health risks. However, currently used methodologies are inadequate for quantifying nanoparticulate exposures, because most of the occupational exposure data is represented in the gravimetric form of mass/volume of sampled air. Our goal is to address this controversial issue with a focus on practicability of measurements under workplace conditions. Therefore it is important to discuss the proposed change in dose metrics for inhalation toxicity studies with engineered aerosolized amorphous silica nanoparticles. Accordingly, rats were exposed to freshly generated nanoparticles at two different particle size ranges. The particle aerosols were characterized by particle numbers using SMPS technology. Our panel of experts will address the merits of using surface area metrics based on measurements of particle numbers and material-specific density for quantifying particle exposures and their use for risk assessment. Our panel will present real-time instrumentation for measuring airborne particle surface area and morphology of nanoparticle agglomerates—two of the important metrics for toxicity evaluation. In addition, recent exposure assessments in manufacturing facilities involving silicon nanoparticles and carbon nanotubes will be presented. It is also important to note the effect of agglomerate structure size on the bioactivity of nanoparticles and present current methods used to disperse nanoparticles for in vitro and in vivo testing which we will address. Finally, we will discuss methods to convert in vivo lung burdens to appropriate in vitro test concentrations of nanoparticles for hazard screening.

IS 2505 PROGRESS OF THE TOX21 CONSORTIUM IN HIGH-THROUGHPUT BIOACTIVITY PROFILING OF CHEMICALS.

R. Kavlock[1] and C. Austin[2]. [1]*U.S. EPA, Research Triangle Park, NC and* [2]*NIH Chemical Genomics Center, Gaithersburg, MD.*

In 2008, the National Institute of Environmental Health Sciences/National Toxicology Program, the NIH Chemical Genomics Center, and the U.S. EPA's National Center for Computational Toxicology entered into a Memorandum of Understanding to collaborate on the research, development, validation, and translation of new and innovative test methods to characterize key steps in toxicity pathways. The U.S. FDA joined this consortium in 2010. A central component is the exploration of high-throughput screening, as well as high-throughput whole genome analytical methods, to evaluate mechanisms of toxicity. The goals of the Tox21 Community are to investigate the use of these new tools, along with existing chemical and biological information, to prioritize substances for further in-depth toxicological evaluation, identify mechanisms of action for further investigation, and develop predictive models for in vivo biological response. Success is expected to result in test methods for toxicity testing that are more mechanistically based and economically efficient; as a consequence, a reduction or replacement of animals in regulatory testing is anticipated to occur in parallel with an increased ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation. In the past year, Tox21 has completed assembly of a library of ~10,000 chemicals and began screening the library against molecular targets and pathways at the rate of one assay per week. Our panel of experts will inform the scientific community of progress in meeting the Tox21 goals, by focusing on the strategies for chemical and assay selection, workflows for data management and analysis, and understanding the human significance of results. The Tox21 effort represents the largest and most comprehensive evaluation of interaction of environmental chemicals with toxicity pathways and is helping to pave the way for the use of high-throughput screening tools in hazard identification, chemical prioritization, and risk assessment.

IS 2506 TOXICOLOGICAL CONSIDERATIONS OF PHARMACOTHERAPY DURING PREGNANCY.

S. Laffan[1], M. Miller[3], C. Buhimschi[4], R. Miller[5], D. Stanislaus[1] and O. Olivero[2]. [1]*Safety Assessment, GlaxoSmithKline, King of Prussia, PA,* [2]*Laboratory of Cancer Biology and Genetics, National Cancer Institute, Bethesda, MD,* [3]*National Center for Toxicology Research, Food and Drug Administration, Washington, DC,* [4]*Obstetrics & Gynecology, Yale University, New Haven, CT and* [5]*Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, Rochester, NY.*

Pharmacotherapy of pregnant women is challenging and requires considerations for the mother and the fetus. Almost every pregnant woman is exposed to some type of medication during pregnancy. Many pregnant women refuse medically important agents or conversely, are prescribed drugs deemed safe despite evidence of possible teratogenicity and/or genotoxicity. An overview of the proposed new pregnancy labeling categories based on reproductive toxicology and present results of pharmacokinetic studies in pregnant women funded by the U.S. FDA Office of Women's Health will be provided. It is therefore important to offer a medical perspective on the challenges involved in treating pregnant and lactating women as a population and as individuals. This process, known as theranostics, will be useful and allow us to tailor medical treatments for individual patients. Because of this process we can focus on maternal pharmacotherapy and role of the placenta in modulation and transfer of agents to the conceptus. In addition, we will be able to examine a range of in vitro assessments for studying the human placenta and animal models as well as investigating maternal and fetal advantages of novel nanomedicines. To fully assess the issue of phamacotherapy, it is important to discuss the unique challenges of supporting clinical trials in pregnant women while developing a novel medicine for preterm labor. Finally, research on the transplacental carcinogenicity of nucleoside analogs and implications on the therapeutic use for HIV will be discussed.

IS 2507 BEYOND SCIENCE AND DECISIONS: FROM PROBLEM FORMULATION TO DOSE-RESPONSE.

M. L. Dourson[1] and R. L. Grant[2]. [1]*Toxicology Excellence for Risk Assessment, Cincinnati, OH and* [2]*Toxicology Division, Texas Commission on Environmental Quality, Austin, TX.*

In follow up to reports released in 2007 and 2008 by the National Academies of Science (NAS), specifically their report Science and Decisions: Advancing Risk Assessment, we will focus our discussion on the approaches for moving the science of dose-response assessment forward. We will present findings and discuss progress from the first and second of three workshops organized with a multi-stakeholder approach to share information, ideas, and techniques in support of developing practical problem-driven risk assessment guidance. The first of these workshops was held in Austin, Texas in March 2010. This workshop highlighted relevant work in issue identification and assessment methods, followed by brainstorming on purpose-focused methods as well as selection of 20 case proposals. The second workshop took place in October 2010 in Crystal City, Virginia, in tandem with the Federal and State Risk Assessment and Toxicology Conference. A third workshop is scheduled to take place in March or April 2011. At this meeting, the case proposal presentations will be evaluated by a yet-to-be identified science panel selected from the second and third workshops. The panel of experts will build consensus on purpose-specific dose-response methods. One of the primary goals of these workshops is to develop practical guidance for the use of risk assessment techniques applicable to specific issue identification (e.g., prioritization, screening and full assessment) and use by risk managers at a variety of levels (e.g., states, regional managers, people in a variety of agencies, and in the private sector). Specific case proposals will be used as examples to identify useful dose-response techniques.

EC 2508 BRINGING TOXICOLOGY TO THE DECISION-MAKERS TABLE: OPPORTUNITIES FOR SCIENCE POLICY AND REGULATORY SCIENCE POSITIONS IN WASHINGTON DC.

N. Beck[1] and M. Mercado-Feliciano[2]. [1]*Office of Management and Budget, Washington, DC and* [2]*NIEHS, Research Triangle Park, NC.*

If toxicologists are truly going to improve and protect public and environmental health, not only do we need to continually be advancing the science, but we also need to bring our expertise and knowledge to the policy makers attention. We will need more toxicologists who can translate the information generated in the laboratory to the policy and regulatory arenas. With the 2011 SOT Annual Meeting being held in Washington, D.C., we are provided with a perfect opportunity to hear from local scientists who have successfully transitioned out of the academic laboratory focused on discovering mechanisms of action into working in policy and regulatory settings that impact public and environmental health. We will highlight the various types of positions and opportunities that exist in and around our nation's capital. Come and learn about what skills and training are most useful to transition from the laboratory to a toxicology policy-maker and regulatory scientist. Our panel of experts include scientists currently doing science policy and regulatory work for a variety of organizations, representing the federal government, private sector, and non-governmental organizations (NGO). Before the interactive roundtable discussion begins, a brief overview summary of the career paths taken, the types of work these scientists are engaged in, and options available for SOT scientists seeking a change will be highlighted.

PS 2509 PREVALIDATION OF LIVER SLICES AS A MODEL FOR THE EARLY ONSET OF LIVER FIBROSIS TO TEST ANTI-FIBROTIC DRUGS.

I. M. Westra[1], G. M. Groothuis[1] and P. Olinga[2]. [1]*Pharmacokinetics, Toxicology and Targeting, University of Groningen, Groningen, Netherlands and* [2]*Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, Netherlands.* Sponsor: A. Vickers.

Liver fibrosis is the progressive accumulation of connective tissue that affects the normal function of the liver and will eventually lead to liver cirrhosis. The aim of this study is to prevalidate precision-cut liver slices (PCLS) as an *in vitro* model to investigate the early onset of liver fibrosis and as a test system for anti-fibrotic drugs. Rat PCLS were incubated up to 48 hours, viability was assessed by ATP content and the gene expression of the fibrotic markers Heat Shock Protein 47 (HSP47), α-Smooth Muscle Actin (αSMA) and Pro-collagen1A1 (PCOL1A1) was determined. Furthermore, during 48 hours the effects of anti-fibrotic drugs inhibiting the PDGF signaling pathway (Gleevec (G), Sorafenib (So) and Sunitinib (Su)) and drugs inhibiting the TGFβ signaling pathway (Valproic acid (Va), Perindopril (Pe)) and drugs that directly affect the collagen expression (Colchicine (Co), Pirfenidone (Pi) and Tetrandrine (Te)), all used at a non-toxic concentration, were determined. In PCLS incubated up to 48 hours an increased gene expression of HSP47, αSMA and PCOL1A1 was found. Addition of G, So or Su resulted in an inhibition of the gene expression of 60, 75 and 80% (G), 35, 60 and 70% (So) and 60, 80 and 90% (Su) of HSP47, αSMA and PCOL1A1 respectively. However, only the highest concentration of Va inhibited the gene expression of the fibrotic markers by 60%. Pe inhibited the gene expression of PCOL1A1 only at the highest concentration by 20%. Co inhibited gene expression of αSMA and PCOL1A1 by 50%, while Pi caused an inhibition of the gene expression of HSP47 (30%), αSMA (60%) and PCOL1A1 (70%) and Te caused only an inhibition of the gene expression of PCOL1A1 by 25%. Rat PCLS incubated for 48 hours show characteristics of early onset of liver fibrosis. This effect is inhibited by anti-fibrotic drugs acting directly on collagen and on the PDGF-pathway, but not on the TGFβ pathway, indicating that PCLS are a model for testing such anti-fibrotic compounds *in vitro*.

PS 2510 NEURONAL NETWORKS COUPLED TO MEA FOR *IN VITRO* NEUROTOXICITY TESTING: RESULTS FROM A RING TRIAL.

B. Scelfo[1], A. Novellino[2], A. Price[1], T. Palosaari[1], T. Sobanski[3], G. Gross[4], T. J. Shafer[7], A. F. Johnstone[7], A. Gramowski[5], O. Schroeder[6], F. Benfenati[8], M. Chiappalone[8], S. Martinoia[8], B. Tedesco[8] and M. Whelan[1]. [1]*IHCP, JRC, Ispra, Italy,* [2]*ETT S.r.l., Genova, Italy,* [3]*ECHA, Helsinki, Finland,* [4]*University of North Texas, Denton, TX,* [5]*University of Rostock, Rostock, Germany,* [6]*NeuroProof GmbH, Rostock, Germany,* [7]*U.S. EPA, Research Triangle Park, NC and* [8]*Italian Institute of Technology, Genova, Italy.*

Neurotoxicity (NT) evaluation represents a major challenge for the registration and risk assessment of chemicals because: a) NT effects can only be identified with in vivo studies; b) no in vitro methods for NT assessment have been validated. Neuronal activity is the output of the nervous system and deviations from its resting level may indicate toxicity. Cultured neurons coupled to microelectrode arrays (MEAs) represent an optimal model for investigating NT in vitro. The MEAs are non- invasive and provide a simpler approach and higher throughput than conventional electrophysiology techniques. Although MEAs have been used to study the action of a variety of chemicals, there have been no studies evaluating the potential of this method for formalized toxicity testing. We set up a ring trial with six independent laboratories. Three reference chemicals: Fluoxetine (FLU), Muscimol (MUS) and Verapamil (VER) were studied. For each chemical descriptors of neuronal electrophysiology have been measured: number of spikes/bursts, mean spike rate, mean burst rate, number of spikes in a burst, burst duration and interburst interval. The results obtained show good intra- and inter- laboratory reproducibility. The inhibitory effects on electrical activity were observed with the following IC50s (mean±S.E.): 2.54±0.21μM for FLU, 0.15±0.50μM for MUS and 4.26±0.83μM

for VER. This outcome suggests that the MEA approach provides accurate and reproducible results. As a further step in the study a larger set of compounds is included for NT assessment and the submission of the MEA technology for validation as standard NT testing method is forecasted.

PS 2511 IDENTIFICATION OF HUMAN HEPATOTOXICANTS BY HIGH-CONTENT IMAGING IN MICROPATTERNED HUMAN HEPATOCYTE COCULTURES.

R. A. Kemper[1] and S. Khetani[2]. [1]*Integrative Toxicology, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT and* [2]*Research and Development, Hepregen Corporation, Medford, MA.*

Drug-induced liver injury (DILI) is the leading cause of acute liver failure and a major driver of drug withdrawals, non-approval decisions and use restrictions/warnings. DILI is often poorly predicted by traditional nonclinical toxicology studies, and there is a strong desire to develop predictive in vitro models for DILI based on human tissue sources. Coculturing human hepatocytes with mouse 3T3-J2 cells in a micropatterned format has been shown to result in long term (2-3 weeks) viability and maintenance of physiologically relevant levels of drug metabolizing enzyme (DME) and transporter activity, making this an attractive system for investigation of DILI. In the current work, we investigate the effects of known human hepatotoxicants and non-hepatotoxicants in micropatterned human hepatocyte cocultures using automated multispectral fluorescence imaging technology to monitor perturbation of intracellular indicators of hepatotoxicity. Microscale architecture was optimized to facilitate efficient imaging of hepatocytes without compromising longevity or DME activity. Micropatterned hepatocyte cocultures are maintained for 5-7 days to allow stabilization of DME activity, then treated with test compounds for 24 hrs at concentrations up to 100X the clinically therapeutic Cmax. Cultures are then stained live with Draq 5 (nuclei), monochlorobimane (GSH), TMRM (mitochondrial membrane potential) and CM-H2DCDFA (reactive oxygen species) and analyzed using a Cellomics Arrayscan VTI. In proof of concept experiments, compounds with well established mechanisms of toxicity showed expected changes in the targeted parameters. Imaging data on a validation set of pharmaceutical compounds suggests that the model can distinguish hepatotoxicants from non-hepatotoxicants, and may show improved sensitivity over previously published human hepatocyte culture models.

PS 2512 DEVELOPMENT OF AN INTEGRATIVE APPROACH FOR THE PREDICTION OF SYSTEMIC TOXICITY: COMBINATION OF CELL TOXICITY, PHARMACOLOGICAL, AND PHYSICAL CHEMICAL PROPERTIES.

R. R. Note[1], H. Noçairi[1], M. Thomas[1], L. Bourouf[1], J. M. McKim[2], G. Ouédraogo[1] and J. Meunier[1]. [1]*L'Oréal, Aulnay Sous Bois, France and* [2]*Ceetox, Kalamazoo, MI.* Sponsor: H. Toutain.

Economical, ethical and scientific constraints have motivated the scientific community to develop alternatives to animal testing. For chronic and systemic toxicity testing, which consume a large number of laboratory animals, no alternatives are available. Developing alternatives in this area represents a challenge because of the complex biological processes implied. A realistic approach could rely on the combination of data generated for multiple endpoints. The Ctox panel® which is a multiparameter, cell-based in vitro system for predicting rat acute systemic toxicity is a typical example. Preliminary studies conducted in a blinded manner indicated that the method had good sensitivity and specificity (91% and 78%) while defining a LD50 threshold at 2000 mg/kg. However, the model failed to accurately predict very toxic chemicals displaying a LD50 value below 300 mg/kg. In order to improve the algorithm, an in depth review and analysis of the false negative and false positive predictions was performed. Conclusions drawn implied considerations of both pharmacological (for the reduction of false negatives) and physical-chemical properties (for the reduction of false positives). The modified approach was applied to 76 non-proprietary compounds previously tested with that statandard method. The predictive performance of the model for the Globally Harmonized System (GHS) categories, was assessed. A significant improvement in the prediction of the GHS categories was observed. Indeed, 75% of the chemicals pertaining to GHS 1, 2 and 3 were correctly classified, compared to 50% with the standard model. In addition, at an LD50 threshold of 500 mg/kg, the sensitivity and specificity were 85% and 89% with the new model against 71% and 83% with the standard model. Future directions will consist of challenging the newly built model with a new set of chemicals.

PS 2513 EVALUATION OF SENSCEETOX® AN INTEGRATIVE MODEL FOR IDENTIFYING CHEMICAL SENSITIZERS.

A. Del Bufalo[1], R. Note[1], S. Teissier[1], C. Gomes[1], C. Piroird[1], J. Ovigne[1], J. M. McKim[2] and J. Meunier[1]. [1]*L'Oréal, Aulnay sous Bois, France and* [2]*Ceetox, Kalamazoo, MI.* Sponsor: H. Toutain.

Allergic contact dermatitis is the result of an adaptive immune response of the skin to direct exposure to an allergen. Because it is the most common manifestation of immunotoxicity in humans, a strict screening of all ingredients in consumer products is required. Current methods rely on animal testing (e.g. local lymph node assay) for determining chemical sensitization. Ethical concerns and regulatory changes in the EU have stimulated the development of alternative tests for the assessment of potential sensitizers. Several in vitro predictive assays have been developed and there is a common view that a strategy for the integration of the available methods will be required. Ceetox Inc. has developed an integrative approach, namely SensCeeTox® allowing the EC3 value to be estimated and differentiating the degree of response from non-sensitizer (NS), weak (W), moderate (M), and strong (S), up to extreme (E). The purpose of this study was to evaluate the predictive capacity of this approach in a blinded manner. L'Oréal provided a set of 40 compounds (20 positive and 20 negative), consisting of 24 proprietary and 16 public domain chemicals that were assessed by CeeTox. All 40 compounds could be classified; those placed into E, S, and M were considered positive, while compounds classified as W or N were considered negative. Accuracy was 72%, with a sensitivity of 71% and a specificity of 74%. However, the model failed to accurately predict each sensitization category. Refinements and automation of the algorithm and the incorporation of additional assays should improve the models' ability to predict potency.

PS 2514 DETERMINING THE EFFECTS OF GLUTATHIONE ON DEVELOPMENTAL METHYLMERCURY EXPOSURE.

R. H. Klingler and M. J. Carvan. *GLWI, University of Wisconsin-Milwaukee, Milwaukee, WI.*

Human exposure to methylmercury (MeHg) comes primarily from consuming contaminated fish. It is well established that prenatal MeHg exposure causes impaired neurological development and results in defects ranging from severe cerebral palsy to subtle deficiencies in learning and memory. Our lab has previously shown that developmental exposure of zebrafish embryos to low doses of MeHg results in persistent neurological defects similar to those seen in humans. MeHg is eliminated from the body as a glutathione (GSH) conjugate. Recent epidemiological studies have identified polymorphisms in genes associated with GSH synthesis or conjugation, which result in elevated blood mercury levels in the carriers. We hypothesize that zebrafish with reduced GSH elimination capacity will also have increased blood mercury levels and will exhibit signs of increased toxicity and neurobehavioral defects in response to developmental MeHg exposure. In this study, we used translation-blocking morpholino oligonucleotides (MO) to knockdown protein expression of four GSH-related genes (glutamate-cysteine ligase catalytic subunit (gclc), glutamate-cysteine ligase modifier subunit (gclm), and glutathione S-transferase pi 1+2 (gstp1+2)). Control MO constructs and those designed against gclc, gclm, and gstp1+2 were injected into the yolk of 1-8 cell stage zebrafish embryos. Immediately following MO injection, embryos were exposed for 24 hours to 0, 0.03, 0.1, or 0.3μM MeHg (as MeHg chloride). Embryos were then observed daily for developmental abnormalities resulting from exposure to MeHgCl. Our results suggest that a lack of gclc expression leads to an embryonic phenotype. In addition, we have found that reduced expression of gclm or gstp1+2 results in an increased MeHgCl sensitivity, as shown by increased morphological defects and mortality when compared to embryos injected with a control MO. These data suggest that individuals with reduced GSH function have increased susceptibility to MeHg toxicity and that our zebrafish model can be utilized to elucidate the relationship between GSH and developmental MeHg toxicity.

PS 2515 DEVELOPMENT OF AN INTEGRATIVE APPROACH FOR THE PREDICTION OF SYSTEMIC TOXICITY: COMBINATION OF CELL TOXICITY AND METABOLISM DATA.

M. Thomas, R. R. Note, J. Eilstein, D. Duché, G. Ouédraogo and J. Meunier. *L'OREAL, Aulnay Sous Bois, France.* Sponsor: H. Toutain.

Increasing societal concerns for animal welfare and current legislation constraints have made the industry enter a new phase in its innovation and R&D processes. For acute and chronic toxicity testing, which consumes a large number of laboratory animals, no alternative is available, one of the reason being the complexity of the biological processes involved. As such, many research programs have been

launched with the aim of developing integrative approaches that would accurately predict such an endpoint. The purpose of the study presented herein was to develop an integrated testing strategy on the basis of the data previously generated with The Ctox panel®, a multiparameter, cell-based in vitro system and the Solidus® DataChip/MetaChip platform, developed to assess metabolism-mediated toxicity. The set used consisted of 63 proprietary chemicals, categorized as Toxic (25 compounds) and Non Toxic (38 compounds) on the basis of a LD50 threshold of 500 mg/kg in rat following an oral administration. A statistical analysis of the data led to the construction of several integrative models. The most predictive model (discriminating analysis) required the consideration of a total of 22 parameters. On the basis of a LD50 threshold of 500 mg/kg, the sensitivity and specificity of the prediction was 92% and 87%. The next steps will consist of challenging the model with a set of diverse chemical classes. At last, it is noteworthy that the number of parameters considered for the model correlates well with the complexity of the endpoint mentioned above.

PS 2516 LIVER-SPECIFIC FUNCTIONS AND EPIGENETIC STATUS OF HEPARG UNDER VARIOUS CULTURE CONDITIONS.

N. Kramer, C. Klotz, J. van den Brink and B. Blaauboer. *Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands.*

A possible alternative to laboratory animals in metabolic clearance studies is the use of human liver cell lines. Unlike many other human liver cell lines, the relatively new HepaRG cell line expresses a majority of human biotransformation enzymes. They have been characterized as progenitor cells that differentiate into adult hepatocytes after two weeks of exposure to 2% dimethyl sulfoxide (DMSO). The aim of this study was to optimize the culture of HepaRG to enhance the cell line's liver specific functions. HepaRG and HepG2 were cultured as 2D monolayers and as 3D cultures using polystyrene scaffolds, the Puramatrix hydrogel, and alginate sponges. The epigenetic status of the cells was assessed and manipulated. 2D and 3D cultured HepG2, undifferentiated HepaRG and differentiated HepaRG were exposed to Trichostatin A (TSA), a histone deacetylase inhibitor, and 5-Aza-2′-deoxycytidine (5-aza-DC), a DNA methyltransferase inhibitor. Analysis of global DNA methylation levels, qRT-PCR analysis of CYP1A1, CYP1A2, CYP2B6, CYP3A4 and albumin mRNA levels, and CYP1A1, CYP1A2, CYP2B6 and CYP3A4 function and albumin secretion assays were performed. Unlike HepG2, the liver-specific functions of particularly undifferentiated HepaRG cells generally improved when the cells were grown in 3D cultures and, to some extent, these improvements coincided with a decrease in global DNA methylation levels. Exposure to TSA and 5-aza-DC further improved the expression and function of cytochrome P450s and the secretion of albumin. These results suggest that HepaRG are amenable to epigenetic manipulations to improve their hepatic function.

PS 2517 DEVELOPMENT OF A *CAENORHABDITIS ELEGANS* MOVEMENT ASSAY FOR ASSESSING NEUROTOXICITY.

J. H. Freedman, D. W. Snyder, W. A. Boyd and G. E. Kissling. *NIEHS, Durham, NC.*

Government agencies are using alternative toxicological model organisms to screen and prioritize chemicals to be used in traditional mammalian studies. The nematode Caenorhabditis elegans is a useful alternative model to characterize the behavioral effects of chemicals, because they possess a simple, well-characterized neuromuscular system. A movement assay has been developed to quantify C. elegans locomotion using a semi-automated motion tracking system. This system consists of an inverted fluorescence microscope equipped with a CCD camera, incubated motorized stage, and image analysis software. Using this system, a number of locomotion parameters were defined including curvilinear velocity, linearity, and amplitude of sinusoidal movement. RNA interference of a number of genes known to control normal nematode movement was used to evaluate the C. elegans movement assay. Changes in specific locomotion parameters indicated that the assay was capable of characterizing various loss-of-function phenotypes, which included uncoordinated and slow movement. A transgenic C. elegans strain expressing a mutant human tau protein, which is associated with neurodegenerative diseases such as Alzheimer's, was also analyzed. With the transgene expressed in all neurons, the mutant C. elegans moves slower than wild-type nematodes. Expression of the mutant tau transgene resulted in significantly impaired C. elegans movement, as determined by various motion parameters. The phenotype was rescued by six different genetic suppressors. Finally, the effects of three chemicals, cadmium, chlorpyrifos and tebuconazole, on C. elegans movement were quantified. All movement parameters significantly decreased by 1 mM cadmium and 0.1 to 10 μM chlorpyrifos. Tebuconazole, however, did not affect nematode locomotion at the highest concentration tested (1 mM). Together, these results confirm the utility of the C. elegans movement assay as a robust endpoint for investing the neurotoxicity of chemicals for toxicant screening and characterization.

PS 2518 DEVELOPMENT OF AN *IN VITRO* DEVELOPMENTAL NEUROTOXICITY (DNT) SCREENING ASSAY USING THE HUMAN NT2/D1 CELL LINE.

M. A. Taylor[1, 2], L. Kan[1] and M. S. Marty[1]. [1]*The Dow Chemical Company, Midland, MI and* [2]*Ashland Chemical Company, Dublin, OH.*

Traditional approaches for DNT testing are labor, time and animal-resource intensive; thus, *in vitro* screening assays that target critical neurodevelopment processes would be useful to prioritize test compounds. This assay was developed to evaluate potential toxicant effects on neuronal differentiation. Cultured NT2 cells were treated with 10 μM all-trans retinoic acid (RA) to initiate neuronal differentiation (RA day 0). Cells were exposed to test compounds for 48 h (RA days 2-4) and on day 9, a single cell suspension was prepared. Cells were fixed, then stained with an Alexa Fluor® 488-conjugated antibody for beta tubulin-III (TUJIII), a marker specific for differentiated neurons. Cell suspensions were analyzed by flow cytometry. Cell metabolism (MTT) and membrane integrity (LDH) were assayed on RA day 4. While TUJIII expression was detectable starting by RA day 7, ideal fluorescent separation of differentiated and undifferentiated cells was noted beginning at RA day 9. RA removal experiments showed that RA days 2-4 were the most sensitive for inducing changes in differentiation rate. For response characterization, the positive control compound, 9-cis RA, which alters all-trans RA signaling, significantly decreased NT2 differentiation at ≥30 μM, a concentration that did not induce cytotoxicity. To verify specificity, N-(4-hydroxyphenyl)retinamide, an apoptotic agent, induced cytotoxicity at a lower concentration than that affecting cell differentiation. 22(R)-hydroxycholesterol, which reportedly induces NT2 differentiation, did not alter differentiation at concentrations up to 25 μM, where slight cytotoxicity was seen. A known developmental neurotoxicant, methylmercury, significantly decreased differentiation at 2.5 μM. At this concentration, slight cytotoxicity was observed with the LDH assay, whereas MTT was unchanged. Thus, the NT2 differentiation assay shows promise as part of a screening battery to prioritize potential DNT compounds, but additional work is needed to further characterize this assay.

PS 2519 A NOVEL *IN VITRO* MODEL OF HUMAN INTRAEPITHELIAL NEOPLASIA.

L. Allen-Hoffmann, E. Gill, M. Pickart, L. Shapiro, N. Peters, C. Rasmussen, C. Krueger and M. Hoffmann. *UW Madison, Madison, WI.*

Over half of all human cancers originate in stratified squamous epithelia. The specific contributions of the surrounding tissue microenvironment to development or progression of primary carcinoma are poorly defined, yet may be important determinants in successful treatment strategies. A model that enables evaluation of tumorigenic cells within the context of the normal surrounding human epithelia is needed to identify chemopreventative agents that block progression of a benign tumor into an aggressive, malignant neoplasm. We have developed an *in vitro* model of intraepithelial neoplasia to provide a biologically relevant, human cell-based screening tool for the assessment of cytotoxic or cytostatic agents. This model incorporates fluorescent squamous cell carcinoma (SCC) cells into a three-dimensional organ-specific microenvironment, enabling sensitive fluorescence-based measurement and quantification of intraepithelial neoplastic growth in response to treatment with chemotherapeutic agents used either systemically or topically.
Fluorescence and confocal microscopy confirmed the formation of fluorescent tumor foci within the normal tissue microenvironment, mimicking *in vivo* SCC tumor growth. Indirect immunofluorescence was employed to further characterize the model. Although absent from the tumor foci, the differentiation markers keratin 1, filaggrin, and transglutaminase-1 were appropriately expressed and localized within the normal tissue microenvironment. Cell membrane-restricted expression of E-cadherin was observed between all cell types. Tumor burden in the model was assessed using multiphoton imaging and an image processing strategy based on the optical contrast of the fluorescent SCC cells. As expected, the total tumor volume increased over time. The percent tumor burden also increased over time, indicating that tumor cell expansion was not accompanied by a concomitant increase in tissue volume. These results support use of this model in the development of novel screening technologies for the determination of anti-cancer therapy efficacy and surrounding normal tissue toxicity.

PS 2520 DIFFERENTIAL EFFECT OF CHLOROPYRIFOS ON EPIGENETIC MARKERS IN PROLIFERATING AND DIFFERENTIATING HUMAN NEURAL STEM CELL.

H. Kim, X. Yu, S. Hong, J. Park and E. M. Faustman. *DEOHS, University of Washington, Seattle, WA.*

Chlorophyrifos (CP), a widely used OP pesticide, has been reported to be associated with altered children's neurodevelopment. We established a human neural progenitor cells (hNPCs) model to examine the potential epigenetic effects of CP on

neuronal stem cells during proliferation and differentiation. hNPCs (ENstem A) cells were expanded in serum free Millipore proliferation expansion medium (MEM) and differentiated in HyClone neural differentiation medium (HDM). Cells were incubated for 72 hours in either MEM or HDM with CP (0-20 μg/mL). Dose-dependent decreases of cell viability were observed in both cells cultured in proliferation MEM and differentiation HDM medium, with significantly greater effects of CP on cells in HDM (78%+/-3.2%) than in the MEM (85%+/- 4%) at concentrations of CP 20ug/mL. Cells grow under differentiating conditions were more sensitive to CP. Protein expression of 18 epigenetic markers including 6 acetyl Histone H3 antibodies, 6 methyl Histone H3 antibodies and 6 HDAC antibodies were examined. We observed dose-dependent increases in phospho-Histone H3 (Ser10) in differentiating cells (about 5 fold increase at 10μg/ml), with no changes in proliferating cells. Significant increases in Acetyl-Histone H3 (Lys18) were shown during proliferation, but no significant changes were observed in this epigenetic marker during differentiation. Dose-dependent increases in di-methyl-histone H3 (Lys79) were shown during differentiation with less change during proliferation. Our results suggest that CP exposure in differentiating hNPCs resulted in significant alteration in expression of several epigenetic markers associated with transcriptional activation while exposure of cells to CP during proliferation showed fewer changes in these epigenetic factors. Differential alterations of epigenetic markers during proliferation and differentiation may provide cell stage specific mechanistic clues for examining effects on neural development.Supported by OCE-0434087, P01ES009601, P50ES012762 and P30ES07033 and RD-83170901.

PS 2521 *IN VITRO* ALGIMATRIX™ 3D CELL CULTURE SYSTEM, AN ALTERNATIVE TO MAMMALIAN MODELS FOR NON-SMALL CELL LUNG CANCER.

M. Sachdeva[1], A. R. Patel[1], M. Chougule[1] and A. Sams[2]. [1]*Pharmacy, Florida A&M University, Tallahassee, FL and* [2]*Invitrogen Corporation, Carlsbad, CA.*

Objective: Fundamental investigations of human biology and development of therapeutics, commonly rely on 2D cell culture systems in-vitro that do not accurately recapitulate the living tissues. There is growing recognition of the need to incorporate the 3D cell culture paradigm towards invitro toxicology models, reduce animal testing, reduce cost to identify new drug candidates and minimize product development time. AlgiMatrix® sponge (Invitrogen Corporation, CA) is a non toxic and biodegradable material made from alginate that maintains normal cell characteristics and morphology. The main objective of the current study was to appraise the AlgiMatrix cell culture system as a 3D invitro tumor model and to ascertain the anticancer activity of Docetaxel and Doxorubicin. Methods: Cell titration was done to optimize seeding density and to determine the optimal incubation time for the formation of spheroids. On day 4, 9 & 13, the number and average size of the spheroids were determined using inverted phase contrast microscopy and imaging software. Further optimized cell density was used to determine the IC50 & IC90 values of drugs. The alamarBlue® assay was performed to determine the number of viable cells in control & treatment wells. Results: Optimized seeding density and incubation time was found to be 0.15 & 0.25 million H460 cells/well and 14 days respectively. Total number of cells obtained by alamarBlue® assay on 14th day was 38.64 ± 4.94 & 42.92 ± 3.29 million cells/well for 0.15 & 0.25 million seeded cells respectively for 6 well plate. Total number of cells obtained by alamarBlue® assay on 14th day was 70.86 ± 9.25 & 117.83 ± 14.37 thousand cells/well for 15 & 25 thousand seeded cells respectively for 96 well plate. The IC50 & IC90 concentration of the docetaxel was found to be 193.19 μM & 290.47 μM when ascertained with 0.15 million cells/well. Conclusion: These results strongly support that the AlgiMatrix 3D Cell Culture System may be used as an in-vitro tumor model and to evaluate cytotoxicity of anticancer drugs.

PS 2522 MICROPATTERNED PRIMARY HEPATOCYTE CO-CULTURES FOR DRUG METABOLISM AND TOXICITY STUDIES.

S. Khetani, A. Moore, S. Krzyzewski, J. Gaffney, C. Kanchagar, J. Shi and J. McGeehan. *Research and Development, Hepregen, Medford, MA.* Sponsor: D. Keller.

Primary hepatocytes display a precipitous decline in phenotypic functions when cultured in a sandwich of extracellular matrix proteins (i.e. collagen, Matrigel). We describe a human liver model with precise microscale cytoarchitecture and optimal stromal interactions (micropatterned co-cultures) that displays stable functions for several weeks in vitro. Micropatterned co-cultures were coupled with miniaturization strategies (i.e. 24- and 96-well format) and optimized for the screening of genotype-specific and clinically- relevant drug disposition and coupled DILI using standard end-points (i.e. ATP depletion, mitochondrial activity) as well as high content imaging. We have investigated the toxicity of several hepatotoxins (i.e. Troglitazone, Tolcapone) in our model under both acute (hours to days) and chronic (weeks) dosing regimens and show concordance with preclinical and clinical findings. Since metabolism is an important determinant of the overall disposition of drugs and the profile of metabolites can have an impact on efficacy and safety, the utility of micropatterned co-cultures for prediction of compound clearance and generation of human metabolites was evaluated. Micropatterned co-cultures classified compounds based on rates of clearance and generated metabolites arising from both phase 1 and 2 reactions (single and sequential). Transporter assays (uptake and efflux) could be performed on micropatterned co-cultures towards simultaneous assessment of the interplay of drug transport, metabolism and toxicity. Long-term drug-drug interaction studies (i.e. enzyme induction and inhibition) have also been explored and results indicate that micropatterned co-cultures are able to recapitulate clinical outcomes. In the future, miniaturized micropatterned co-cultures may find utility in the development of several classes of therapeutic compounds (drugs, biologics), in evaluating the injury potential of environmental toxicants, in fundamental investigations of liver physiology, and in personalized medicine for liver disease.

PS 2523 ASSESSING CHRONIC TOXICITY OF FIALURIDINE IN A MICROPATTERNED HEPATOCYTE CO-CULTURE MODEL.

S. Krzyzewski[1], S. Khetani[1] and S. Barros[2]. [1]*Research and Development, Hepregen, Medford, MA and* [2]*Alnylam Pharmaceuticals, Cambridge, MA.*

Fialuridine (FIAU), a nucleoside drug for the treatment of hepatitis B, failed in clinical trials due to mitochondrial toxicity. FIAU toxicity is difficult to replicate in sandwich cultures of primary hepatocytes as their phenotypic functions display a precipitous decline. We have utilized microfabrication tools to develop a human liver model with precise microscale cytoarchitecture and optimal stromal interactions (micropatterned co-cultures) that displays phenotypic stability for several weeks in vitro. Micropatterned co-cultures from 2 donors were dosed twice over 4 days with FIAU, uridine and 4 other related drug analogues up to 100 uM. Human hepatocytes in this model experienced significant dose- and time-dependent toxicity with FIAU incubation as assessed by mitochondrial activity, morphology, albumin secretion, urea synthesis and CYP3A4 activity. FIAU was the most toxic to human hepatocytes as compared to the other analogues when all endpoints were considered. There were donor dependent differences in the magnitude of toxicity as well as the rank ordering of the compounds. On the other hand, rat hepatocytes did not exhibit the same extent of toxicity upon FIAU incubation as did human hepatocytes in micropatterned co-cultures, consistent with preclinical animal toxicity data. We then dosed micropatterned co-cultures with either human or rat hepatocytes with fresh compounds in culture medium every 2 days for 3 weeks at doses up to 10 uM. Our results indicated that CYP3A4 activity was the most sensitive functional marker as compared to albumin secretion and urea synthesis to distinguish the effects of FIAU over the other drugs at doses as low as 1 uM. In the rat model, urea secretion was down-regulated by as much as 50% with FIAU and other compounds upon chronic administration. Mechanistic investigations are ongoing to distinguish the species-specific effects of FIAU in micropatterned co-cultures. In the future, micropatterned co-cultures may serve as a robust model system to evaluate the chronic effects of compounds on the liver.

PS 2524 TRANSCRIPTIONAL INDUCTION OF ALDO-KETO REDUCTASES (AKRS) BY BENZO[A]PYRENE IN JURKAT CELL LINE.

B. S. Barrón-Vivanco[1, 3], E. González-Barbosa[3], I. M. Medina-Díaz[3], A. E. Rojas-García[3], S. Rothenberg[1], G. Elizondo[2] and A. Albores[1]. [1]*Toxicology, Cinvestav-Ipn, México, Mexico,* [2]*Cell Biology, Cinvestav-Ipn, México, Mexico and* [3]*Laboratorio de Contaminación y Toxicología Ambiental, Universidad Autónoma de Nayarit, Tepic, Nayarit, Mexico.*

Polycyclic aromatic hydrocarbons (PAHs) are chemicals formed during the incomplete combustion of carbon and are carcinogenic to humans. To exert their deleterious effects, PAHs are metabolically activated; one route is through of aldo-keto reductases (AKRs). Benzo[a]pyrene (B[a]P) is considered as the most representative PAH. There are studies in human cell lines such as HepG2 and A549, where AKR mRNA over-expression was observed in response to PAHs exposure. In addition, some human studies have shown the association of AKR mRNA over-expression of some isoforms with some human pathologies like esophagus or bladder cancer and drug resistance. Despite this evidence, studies in open populations are limited because they involved tissue sampling. Thus blood is a good tissue sample candidate because it is easy to obtain and PAHs effects can be readily detected. Therefore, we used Jurkat (lymphocytes T) cell line to assess AKR mRNA levels after PAHs exposure. Jurkat cells were added with human liver S9 fraction to activate B[a]P metabolically and exposed to B[a]P (0.1 - 5.0 μM) for 3, 6, 12 and 24 h. We assessed: cells viability (trypan blue test), AKR1A1, AKR1C1, AKR1C2 and AKR1C3 iso-

forms mRNA expression levels were assessed by quantitative real-time PCR assay (rtPCR). A time-dependent variation of the mRNA expression levels was observed. The maximum expression was detected at 12 h. The observed effect was similar for the four genes studied. B[a]P treatment had no effect on the viability of cells.
This is the first study to our knowledge where lymphocyte cells are used to evaluate the PAHs effect on the AKRs mRNA expression.

PS 2525 DATACHIP/METACHIP 2.0: AN IMPROVED CHIP PLATFORM FOR 3D CELL-BASED TOXICOLOGY ASSAYS.

M. Lee[1], J. Ryan[1], S. Brooks[1], J. Gray[1], D. Lee[2], S. Jeong[2], J. Yang[2], B. Ku[2], Y. Will[3] and S. Nadanaciva[3]. *[1]Solidus Biosciences, Inc., San Francisco, CA, [2]Central R & D Institute, Samsung Electro-mechanics Co., Suwon, Republic of Korea and [3]Compound Safety Prediction, Pfizer Inc., Groton, CT.*

Several studies suggest that 3-D cell cultures are more likely than 2-D cell cultures to reflect accurate physiological responses to cytotoxic compounds. Solidus Biosciences has developed the DataChip/MetaChip platform that couples a 3-D cell culture array with a complementary array of recombinant enzymes to identify metabolism-induced toxicity. The IC50 values of many compounds not only depend on the morphology of the cultured cells, but also vary with culture conditions and the cell type used. Access to a greater number of cell culture conditions on a high-throughput format enables the generation of a larger and more-predictive portfolio of data for new chemical entities. Thus, we have optimized the architecture of both the DataChip and the MetaChip platform for metabolism-induced toxicity measurements to enable the screening of compounds against multiple cell types cultured in 3-D matrices under different conditions on a single chip platform. In the new embodiment of the DataChip, cells are immobilized within a variety of matrices on 532 elevated pillars that are then submersed in microwells of the modified complementary MetaChip. Recombinant enzymes are sequestered within matrices in the microwells to enable the in-situ generation of metabolites. Because each cell culture is immersed in media in discrete wells, multiple cell types requiring different media can be cultured on the same chip. The IC50 values of compounds known to have cytochrome P450 generated toxic metabolites, such as Troglitazone, are compared under different culture conditions for several cell types. These studies demonstrate the increased throughput and capability of the modified MetaChip/DataChip system to identify metabolism-induced toxicity at early stages in compound safety assessment.

PS 2526 HIGH-THROUGHPUT GENE TRANSFECTION ON A THREE-DIMENSIONAL (3-D) CELL MICROARRAY PLATFORM FOR METABOLISM-INDUCED TOXICOLOGY SCREENING.

S. Kwon[1], J. Gray[2], B. Ku[3], J. Ryan[2], D. Clark[4], J. Dordick[1] and M. Lee[2]. *[1]Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY, [2]Solidus Biosciences, Inc., San Francisco, CA, [3]Central R&D Institute, Samsung Electro-Mechanics Co., Suwon, Republic of Korea and [4]Department of Chemical Engineering, University of California Berkeley, Berkeley, CA.*

Variation in metabolic enzyme expression among segments of the human population may cause deviations from the expected pharmacokinetic profile of a drug, resulting in idiosyncratic toxicity or a lack of efficacy. Cell lines that stably express individual or combinations of metabolic enzymes are emerging tools for prediction of these rare clinical events; however, it is difficult to create and maintain a library of stable cell lines that mimics the diversity of metabolic profiles that give rise to unexpected outcomes. In addition, it is difficult to control the expression levels of each of the enzymes in stably-transfected cell lines. To address this need, we have developed a technique for simultaneously infecting an array of miniaturized 3-D cell cultures with varying concentrations of recombinant adenoviruses carrying genes for different metabolic enzymes, which generates an array of cell cultures with differentiated metabolizing capabilities. Compounds can be added to this array of cell cultures to assay the compounds and their metabolites for a variety of end points including, but not limited to, cell viability. We have demonstrated the controlled expression of individual and multiple reporter proteins (GFP and RFP) and drug-metabolizing enzymes (CYP3A4, CYP2C9, and UGT1A4) in two human liver cell lines (HepG2 and THLE-2 cells) on the chip by altering the MOI of the various recombinant adenoviruses. The microarrays of 3-D human cells expressing metabolic enzymes were tested with model compounds, such as acetaminophen, to simulate enzyme-specific hepatotoxicity. These studies demonstrate that the new chip platform can provide critical information necessary for evaluating metabolism-induced toxicity in a high-throughput manner.

PS 2527 CELL TOXICITY AND INTERLEUKIN (IL) 8 PRODUCTION: UNIQUE RELATIONSHIP WITH ALLERGENICITY.

I. Kimber[1], C. F. Portsmouth[1], R. Pendlington[2], G. Maxwell[2] and R. J. Dearman[1]. *[1]University of Manchester, Manchester, United Kingdom and [2]Unilever Safety & Environmental Assurance Centre, Bedford, United Kingdom.*

There has been considerable interest in the development of in vitro tests for the characterisation of chemical allergens, such as the production by dendritic cell (DC)-like cell lines of cytokines. These methods are often limited by toxicity and delivery of chemical allergen in culture. We have constructed toxicity and IL-8 secretion dose response curves for a number of human cell lines (DC-like : THP-1, U937; erthyromyeloid: K562). Cells were incubated for 24h with the contact allergen dinitrochlorobenzene (DNCB) or its water soluble analogue, dinitrobenzene sulfonic acid (DNBS), or with the irritant benzenesulfonic acid (BS). All cell lines expressed IL-8 constitutively and displayed similar toxicity profiles for each of the individual chemicals. DNCB and DNBS enhanced IL-8 production at subtoxic doses (10μM and 300μM, respectively). In contrast, despite using doses that caused similar toxicity profiles to those observed for the allergens, there was no increase in IL-8 secretion recorded for BS (30 to 300mM). Cells (THP-1 and U937) were cultured with two additional allergens (dinitrothiocyanobenzene [DNTB] and dinitrofluorobenzene [DNFB]) or two irritants (1-bromobutane [1BB] and sodium lauryl sulfate [SLS]) at subtoxic and toxic doses. In addition, cells were incubated at varying osmotic pressures (ranging from 0 to 500mOsMo). Treatment with subtoxic doses of DNTB and DNFB (10μM) induced the production of IL-8 by both THP-1 and U937 cells, whereas toxic doses of 1BB and SLS (10μM and 0.3μM, respectively) were without effect. Furthermore, under conditions of hypo-osmolality (100mOsMo) and hyper-osmolality (400mOsMo), both of which caused cell death, there was no marked increase in IL-8 secretion. These data reveal that the relationship between toxicity and IL-8 production is unique to allergens. Defining the signalling pathways involved may allow the identification of novel cellular biomarkers that, compared with IL-8, may offer increased sensitivity and selectivity.

PS 2528 SCREENING DRUG-INDUCED MITOCHONDRIAL TOXICITY USING A HIGH-THROUGHPUT DUAL-PARAMETER CELL BASED ASSAY.

J. Hynes[2], C. Carey[2], S. Kirwan[2], R. Swiss[1] and Y. Will[1]. *[1]Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT and [2]Luxcel Biosciences, Cork, Ireland.*

Recent years have seen a growing appreciation of the importance of the mitochondrion as a site for off-target drug effects. This in turn has prompted the development of a variety of in vitro plate-based mitochondrial toxicity assays, including measuring oxygen consumption in isolated rat liver mitochondria and quantifying the differential ATP depletion rates of aerobically poised versus conventionally cultured HepG2 cells. Both assays identify similar compounds, but each assay also identifies a unique set of compounds. To investigate if a cell-based metabolic assessment could be used to address shed light on this phenomenon, a 384 well assay capable of simultaneous monitoring of both glycolytic flux and oxygen consumption was developed. Cell-based analysis addresses the risk of over prediction which exists with an isolated organelle while also allowing confirmation of mitochondrial dysfunction by observing modulation of both oxygen consumption and glycolytic flux. HL60s were chosen over an adherent cell model as they exhibit similar sensitivity to mitochondrial impairment while also removing the requirement for pre-plating, thereby simplifying workflows. The approach was initially validated using a small compound set and subsequently used to screen a library of 200 compounds across a 10 point dilution series. A small subset of compounds is flagged by all three assays with isolated mitochondrial analysis flagging more than ATP depletion. The vast majority of compounds flagged by either assay are detected by cell-based metabolic analysis; however this approach appears to be more sensitive, confirming metabolic perturbations for a larger number of compounds, albeit typically above 125uM. These data suggest that over-prediction using isolated mitochondria is not a significant problem, but also points to the fact that additional retrospective analysis is required to allow such cell-based metabolic analyses to be correctly interpreted.

PS 2529 USING MULTIPLE ASSAYS TO ASSESS DRUG-INDUCED MITOCHONDRIAL TOXICITY IN EARLY TOXICOLOGY TESTING.

R. Swiss, P. Rana and Y. Will. *Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT.*

Drug-induced mitochondrial dysfunction has been shown to contribute to organ toxicity and late stage attrition. Therefore, testing for drug-induced mitochondrial dysfunction pre-clinically is vitally important. We compared two separate assays to

see whether both were needed or if one assay was sufficient in detecting mitochondrial impairment. The first assay uses isolated rat liver mitochondria and measures a compound's effect on oxygen consumption. The second assay uses HepG2 cells grown in either glucose or galactose containing media. Cells grown in galactose are susceptible to mitochondrial insult, whereas cells grown in glucose are resistant. We tested three sets of compounds that were associated with different organ-specific toxicity. We tested 197 cardiac, 254 hepatic, and 83 nephritic compounds, which included both, Pfizer internal and commercially available compounds.
Of the 534 compounds, 2.5 % flagged positive in isolated mitochondria as well as in galactose grown HepG2 cells, 3.5% flagged in isolated mitochondria and glucose and galactose cells, indicative of additional toxicity mechanisms. To our surprise 5% of the compounds (27 total) only showed toxicity in the isolated rat liver mitochondria assay but not in HepG2 cells. We further analyzed these compounds using the Seahorse platform which measures oxygen consumption rate and extracellular acidification rate of intact cells simultaneously in real time. The mitochondrial impairment was confirmed in the Seahorse experiments when compounds were analyzed in real time. However, time course experiments revealed that cells could recover from the insult and therefore exhibited no frank cell toxicity. In summary, the current results indicate that including both assays in assessing mitochondrial toxicity is important for identifying the greatest number of compounds showing mitochondrial impairment. The mechanism behind the compensatory response warrants additional future mechanistic work.

PS 2530 JC-1 MITOCHONDRIAL MEMBRANE POTENTIAL MEASUREMENTS OF H9C2 CELLS GROWN IN GLUCOSE AND GALACTOSE CONTAINING MEDIA DOES NOT PROVIDE ADDITIONAL PREDICTIVITY TOWARDS MITOCHONDRIAL TOXICITY ASSESSMENT.

P. Rana, Y. Will and S. Nadanaciva. *Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT.*

Drug-induced mitochondrial toxicity has been recognized to be a contributing factor to a variety of organ toxicities. The fact that some, but not all members of a particular drug class can induce mitochondrial dysfunction has necessitated the need to deploy predictive screens within the drug development process. One of these screens is a cell viability assay done in two types of media, one supplemented with glucose and the other with galactose. Since galactose-grown cells are more susceptible to mitochondrial toxicants than glucose-grown cells, this assay distinguishes compounds that cause toxicity primarily through mitochondrial targets from those that cause multifactorial toxicity. However, the assay cannot distinguish compounds that cause toxicity through secondary effects on mitochondria from those that cause toxicity through non-mitochondrial targets. Mitochondrial membrane potential measurements are thought to be indicative of mitochondrial impairment and can be measured in the absence of frank cytotoxicity. Here we investigated if multiplexing mitochondrial membrane potential measurements with the fluorescent dye, JC-1; together with cell viability measurements in glucose and galactose-grown H9C2 cells could provide additional value for compounds that have a multifactorial toxicity. We tested 29 drugs with well characterized mechanistic toxicity profiles. These compounds set included compounds with different therapeutic uses i.e. antidepressants, anti-diabetic agents, anti cancers, etc. The multiplexed assay was able to detect mitochondrial toxicants correctly but did not reveal additional information regarding compounds that exhibited multifactorial toxicity; hence, JC-1 measurements did not provide additional information beyond what was detected using the cell viability assay. Thus, we conclude that even though this multiplexed assay is useful for HTS applications, it provides no additional value over the glucose-galactose cell viability assay.

PS 2531 TREATMENT OF FATTY ACIDS INCREASES THE SUSCEPTIBILITY OF CELLS TO TOXIC COMPOUND INDUCED INJURY.

Y. Luo, P. Rana and Y. Will. *Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT.*

Fatty acids are important source of energy and cells are exposed to various fatty acids under physiological conditions. Excessive energy intake results in elevated levels of fatty acids or triglycerides and cause metabolic disorders such as dyslipidemia, obesity, insulin resistance and diabetes. Several lines of evidence suggest that metabolic diseases increase the susceptibility to drug induced liver injury, cardiotoxicity and renal toxicity. Regular cell culture conditions contain very low levels of fatty acids, much less that than physiological concentrations. It has never been reported whether fatty acids influence the sensitivity of cells to drug induced toxicity. The aim of this study is to investigate the effects of fatty acids on drug induced toxicity. In this study, we tested the effects of palmitate and oleic acid on the viability of HepG2 cells. High concentrations of fatty acid treatment induced cell apoptosis and reduced cell viability. Subsequntly, sub-toxic and toxic concentrations of fatty acid were chosen to investigate whether fatty acids could potentiate drug induced toxicity. We incubated the cells with various concentrations of fatty acids (palmitate and oleic acid) and compounds followed by analyzing cell viability. Here we report the results for statins (simvastatin, cerivastatin, lovastatin, atrovstatin, pravastatin) and glitazones (ciglitazone, troglitazone, rosiglitazone, and pioglitazone). Furthermore, increased population with metabolic diseases pose higher risk for drug induced toxicity, and this assay may identify compounds that have higher risks in this population, individually or in combination therapy.

PS 2532 ER-STRESS AS A CONTRIBUTOR TO HEPATOTOXICITY: DEVELOPMENT OF A SCREENING PARADIGM.

T. Schroeter, L. Qui, M. Pletcher and Y. Will. *Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT.*

The greatest challenge facing the pharmaceutical industry today is developing more effective decision making tools and strategies to reduce the attrition rate of novel compounds during candidate selection and clinical development. Currently, approximately 24 out of 25 compounds fail in clinical trials, a situation that is unsustainable for the industry. One of the major attrition reasons is hepatotoxicity. Gene expression profiles associated with known idiosyncratic hepatotoxicity, revealed ER stress as an important contributor to drug-induced liver toxicity. We developed two assays to investigate whether induction of the endoplasmic reticulum stress response (ERSR) is a predictive reporter of liver toxicity. The first assay detects nuclear translocation of spliced XPB-1 by enzyme fragment complementation (DiscoveRx) and the second assay is a luciferase CHOP reporter assay.
We screened 180 Pfizer internal and commercial available hepatotoxicants and 42 drugs not known to cause organ toxicity in both assays. Compounds were screened in both assays at a final concentration of 150 uM. The XBP-1 screen identified 16 hits. Full dose response curves were generated for all hits and we were able to confirm 13 out of 16 XBP-1 hits. To our surprise, 4 (Ergocalciferol, Dipyridamole, Orphenadrine and Propanolol) of the 13 confirmed XBP-1 hits were drugs with no known organ toxicities. Of the 9 liver toxicants, 7 showed signs of cytotoxicity but so did two of the 4 drugs not known to be organ toxic. The CHOP screen identified 3 hits which could all be validated. All of them are known liver toxicants (Riluzole, Colchicine and Oxybendazole) with Riluzole showing signs of cytotoxicity. Current work centers around confirmation of pathway activity of all active compounds in HepG2 cells to assist in vitro to in vivo correlation.

PS 2533 HIGH-CONTENT IMAGING ASSAY FOR DETECTING ACCUMULATION OF COMPOUNDS IN LYSOSOMES.

S. Nadanaciva[1], S. Lu[2] and Y. Will[1]. [1]*Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT and* [2]*DSRD, Pfizer Global Research & Development, La Jolla, CA.*

Known as the cell's recycling center, lysosomes are essential for the degradation of old organelles, endotoxins and engulfed microbes. Many enzymes which function in the acidic milieu of the lysosome are involved in degradative processes. Compounds which are lipophilic weak bases or lipophilic neutral amines can readily cross the lysosomal membrane from the cytoplasm. Once inside lysosomes, however, these compounds become protonated and are unable to cross the lysosomal membrane to re-enter the cytoplasm. The trapping of compounds within lysosomes can cause alkalinization of these organelles, resulting in lysosomal dysfunction. Hence, high-throughput screens to detect lysosomotropism, the accumulation of compounds in lysosomes, are desirable.
In light of this, we developed a 96-well format multiplexed high content screening (HCS) assay that measures both lysosomotropism and cytotoxicity. The effect of a selection of compounds on these two endpoints was measured in H9c2 cells by using the fluorescent dyes, LysoTracker® Red and Hoechst. Quantitative image analysis showed that lysosomotropic compounds included the antimalarial, chloroquine, antidepressants (sertraline, paroxetine, fluoxetine, imipramine, nortriptyline), antipsychotics (chlorpromazine, thioridazine), antiarrhythmics (amiodarone, propranolol), and some anticancer agents (tamoxifen, imatinib, dasatinib, gefitinib, lapatinib). Although structurally and pharmacologically diverse, compounds which caused lysosomotropism had a CLogP value > 2 and a basic pKa value between 6.5 to 11. In contrast, compounds which did not have this combination of physicochemical properties were not lysosomotropic. The HCS assay that we developed is a robust and rapid method for investigating lysosomotropism and can be implemented within a screening paradigm for identifying drug-induced lysosomal dysfunction.

PS 2534 CUTANEOUS WOUND HEALING IN THE EPIDERM-FT™ FULL-THICKNESS *IN VITRO* HUMAN SKIN MODEL.

P. J. Hayden, A. Armento, C. Cooney, G. Stolper, M. Li, H. Kandarova and M. Klausner. *MatTek Corporation, Ashland, MA.*

Cutaneous wound healing involves interactions between dermal fibroblasts (FB) and epidermal keratinocytes (KC) as well as cell-extracellular matrix interactions. The current study describes wound healing experiments conducted in a full thickness in vitro human skin model (EpiDerm-FT). The model exhibits stratified epidermal components, a well developed basement membrane and in vivo-like barrier function. Small epidermal-only wounds (3mm biopsy punch) or full-thickness wounds (cauterizer burns) were induced in the model and cultures were processed for histological evaluation at various time points to observe the healing process. H&E stained sections of burn wounds showed necrotic epithelium and denatured collagen matrix immediately following burning. Beginning at day 1, matrix degradation and KC migration into the wounded area was observed. Over the course of 7 days, migrating KC covered the wounded area and FB were observed repairing the dermal matrix. Biopsy wound experiments were conducted in growth factor free medium or medium supplemented with 2% human serum (HS). Wounded tissues cultured without growth factors had a reduced healing rate where KC did not cover the entire wound within 6 days. Wounded tissues cultured in 2% HS had a significant increased healing rate with complete wound coverage by day 6. Increased FB proliferation in dermal areas directly adjacent to epidermal wounds was observed in cultures supplemented with 2% HS. Gene expression profiling of the wounded area showed temporally regulated changes in mRNA expression of basement membrane components, collagens and genes involved in extracellular matrix remodeling. FB proliferation and epidermal healing in tissues cultured in 2% HS was severely impaired in the presence of an EGFR tyrosine kinase inhibitor or a TGFα neutralizing antibody. These results demonstrate that EpiDerm–FT is a useful in vitro skin model for investigating dermal–epidermal interactions during wound healing and evaluation of the role of growth factors or new therapeutics in the cutaneous wound healing process.

PS 2535 EVALUATION OF EPISKIN AND EPIDERM SKIN IRRITATION METHODS FOR TESTING SILICON-BASED MATERIALS.

M. L. Jovanovic, J. Arthurton and P. Jean. *Dow Corning, Auburn, MI.*

In vitro skin irritation test methods have been formally validated and adopted within the European Union as an OECD Test guideline. Since silicone-based materials were not included in the validation effort, it is necessary to assess the methods and outcome as it relates to silicon-based materials with properties that may prove difficult to use in the existing protocols. The assay is based on the reduction of cell viability in treated tissues, measured by enzymatic conversion of the vital dye MTT into a blue formazon salt, and expressed as a percentage of the negative control. The cut-off value of percentage cell viability distinguishing irritant from non-irritant is 50%. In vitro data are compared to the in vivo irritation score obtained in rabbits (Draize test). Fifteen silicone materials (12 non- irritants and 3 irritants by Globally Harmonized System classification) from seven different chemical classes (siloxane, polysiloxane, polysiloxane/copolymer, organosilane, silicone resin, silazane and microemulsion) were selected to evaluate the ability of the test method employing reconstituted human epidermis (RhE) models, EpiSkin and EpiDerm, to reliably discriminate skin irritants from non-irritants. However, the lack of silicon-based materials with known irritation potential resulted in an unbalanced distribution of irritation categories. We found that the EpiSkin model under-predicted irritants (33% sensitivity), while the EpiDerm model showed a lower accuracy when identifying non-irritants (58% specificity). The overall accuracy obtained with EpiSkin and EpiDerm was 87% and 67%, respectively. The work presented here provides a platform for further mechanistic and validation efforts and emphasizes the need to test additional silicon-based material irritants to afford a balanced determination of the predictive capacity of these in vitro test methods.

PS 2536 COMPARISON OF FCM AND ELISA IN THE EVALUATION OF THE OF SKIN SENSITIZATION BY NON-RADIOACTIVE MURINE LOCAL LYMPH NODE ASSAY USING BROMODEOXYURIDINE.

Y. Yum[1], Y. Lee[1], K. Jung[2], K. Lim[2], J. Park[1], H. Park[1], J. Kim[1], J. Yang[1], S. Sohn[1], J. Chung[3] and S. Han[1]. [1] *Toxioclogical Evaluation and Research Department, National Institute of Drug and Food Safety Evaluation, Seoul, Republic of Korea,* [2] *Amorepacific Corporation R&D Center, Yougin-si, Republic of Korea and* [3] *College of Pharmacy, Seoul National University, Seoul, Republic of Korea.*

The murine local lymph node assay (LLNA) using H3-thymidine has been developed to detect chemical allergens, replacing the traditional guinea pig maximization test. Recently, non-radioactive local lymph node assay employing bromodeoxyuridine(BrdU) incorporation, LLNA: BrdU-FC method has been developed to extend its utility further. The FCM analysis of BrdU incorporation according to modified MB research's protocol has good sensitivity, based on measuring of individual cellular level. In 2010, non-radioisotopic LLNA using ELISA method was adopted by OECD Test Guideline(OECD TG 442B). In this study, we tried to perform two nonradioactive LLNA tests of reference substances including 3 sensitizer and 1 non-sensitizer, using Balb/c strain mice. We assessed the chemicals in accordance with the decision criteria for positive sensitizer which are stimulation index(SI) ≥1.6, in LLNA:BrdU-ELISA and stimulation index(SI) ≥3, in LLNA:BrdU-FCM.

PS 2537 ADAPTATION OF THE RAT HEPATOCYTES LONG-TERM CULTURE FOR HIGH-CONTENT IMAGING TO PREDICT CHRONIC LIVER TOXICITY *IN VITRO.*

G. Davide, C. Zehnacker, P. Couttet, S. Chibout, O. Grenet, M. Uteng, F. Pognan, M. Dong and A. Wolf. *Translational Sciences/Investigative Toxicology, Novartis Institute for Biomedical Research (NIBR), Basel, Switzerland.*

The conventional Collagen-sandwich culture with the soft/wet gel proved not to applicable to the high-content cellular imaging method due to interferences of the collagen with fluorescence dyes. In addition the Cellomics™Arrayscan® technological platform requires cells to be evenly distributed and orientated on the same plane, since it does not work in confocal mode. To overcome these hurdles MatrigelTM was used instead of Collagen as the top layer. Multiple MatrigelTM administrations were found to be essential to maintain the hepatocyte integrity for 14 days in culture. The optimal conditions achieved were three times application of MatrigelTM on day 1, 6 and 9 to cells seeded on Collagen I-coated plates. Markers of cellular integrity investigated were morphology, cellular ATP content, LDH release and the mRNA expression of several canalicular/basolateral transporters, cytochrome enzymes and nuclear receptors. The secretion of the fluorescent bile acid Choly-lysyl-fluorescein (CLF) by canalicular bile salt excretion pump (BSEP) was used as functional marker. CLF secretion rates were stable and similar during 14 days. Inhibition experiments with the BSEP inhibitor Cyclosporine A (CsA) at day 5, 9 or 14 of the culture indicated no quantitative differences in terms of the inhibitory CsA concentrations. These results demonstrated that the BSEP kept its function for 14 days under the current culture conditions. In conclusion, the further development of the rat hepatocyte long term culture allows the application of high content microscopy imaging, which may serve as powerful tool to investigate chronic liver toxic drugs under in vitro long-term incubation conditions.

PS 2538 AN *IN SILICO* APPROACH FOR COMPUTING OCCUPATIONAL EXPOSURE LIMITS OF ORGANIC SOLVENTS.

M. Debia and K. Krishnan. *Département de Santé Environnementale et Santé au Travail, Université de Montréal, Montréal, QC, Canada.*

Health-based occupational exposure limits (OELs) are derived either on the basis of human observations or from animal point of departures. In the absence of relevant human or animal data for identifying the point of departure, it is a real challenge to derive or propose an OEL. The objective of this study was to develop an in silico approach for computing OELs in data-poor situations. Quantitative Property-Property Relationship (QPPR) approaches were developed using a database of 88 solvents which have health-based Time-Weighted Average (TWA) OELs published by the American Conference of Governmental Industrial Hygienists (ACGIH). Three surrogates of biotic lipid:air partition coefficients [n-octanol:air (Koa), olive oil:air (Koila) and fat:air (Kfa)] were selected for evaluating the descriptive/predictive relationship with OELs for solvents with local modes of action. Koa is the most accurate predictor of OELs [OEL (ppm) =10xEXP((-0.45*logKoa)+3.65); n=21; r^2=0.71; PRESS/SSY=0.36; F=45.5 with p<0.001] and the mean (±SD) (range) of the recommended to predicted OELs was 1.04 ± 0.61 (0.2-2.5). For solvents with systemic modes of action, the prediction of OEL considered quantitative differences in the toxicokinetic and toxicological potency (i.e. effective internal concentration, EIC). Kow is an accurate predictor of calculated EICs [EIC (μmol/L) =10xEXP((-1.16×logKow)+3.65); n=27; r^2=0.88; PRESS/SSY=0.12; F=181 with p<0.001] and 50% of the predicted OEL values were within a factor of two of the recommended TWA OELs. The animal-alternative algorithms developed in this study represent potentially useful tools for estimating provisional OELs for solvents lacking such guideline values. These provisional OELs can be used as an initial measure of relative OEL values rather than absolute values of OEL of chemicals to guide the hygienist in making pragmatic decisions for managing occupational health hazards [Supported by AFSSET/NSERC]

PS 2539 PREDICTION OF HEPATOTOXICITY IN ZEBRAFISH.

C. Callol, C. Quevedo and A. Letamendia. *Biobide S.L., San Sebastian, Spain.*
Sponsor: S. Tripathi.

Drug-induced hepatotoxicity is one of the most important causes of drug withdrawal in the process of Drug Discovery. The low correlation of the in vitro cytotoxicity studies, with human hepatotoxicity, especially in the early stages of Drug Discovery, make human hepatotoxicity difficult to predict. Therefore, the finding of new methods and models to predict hepatotoxicity is a growing area of research. The zebrafish model has been extensively studied in the last decades for its ability to regenerate. Specifically, the liver is one of the organs with the highest regenerative capacity and research over the past 10 years have characterized many of the mechanisms that occur during this process leading to an important knowledge of the signaling pathways that govern zebrafish liver function and development.
Therefore, because of the functional and development similarities between the liver of zebrafish and mammals, we propose to analyze the potential of zebrafish for prediction of hepatotoxic agents. For this purpose, the following aspects have been studied: i) The stage at which adult liver markers are expressed has been identified. ii) Several techniques have been developed for assessing the hepatotoxicity of a compound in zebrafish. iii) These techniques will be validated by testing different drugs. iv) The results will be compared with those obtained in mammals.
Our results indicate that zebrafish embryos could be used as an alternative model in the Drug Discovery process complementing the information of in vitro models and mammals and providing pharmaceutical industry of new assays which might decrease the current huge gap between in vitro and in vivo assays.

PS 2540 PREDICTION OF DRUG-INDUCED LIVER INJURY WITH HIGH SENSITIVITY AND SPECIFICITY WITH HIGH-CONTENT CELL-BASED IMAGING.

R. N. Ghosh, S. J. Hong, B. A. Samson, K. Corkan, M. Pietila, C. Vasudevan and J. R. Haskins. *Thermo Fisher Scientific, Pittsburgh, PA.*

Drug-induced liver injury is a major reason for drug failure and withdrawal from the market. An assay system that enabled early and accurate prediction of drug-induced hepatotoxicity with high sensitivity and specificity would benefit drug development by improving the safety profile of newly developed drugs. Developing an in vitro assay that accurately predicts the toxicology of drug candidates remains a challenge in drug development. A critical factor contributing to the predictive nature of such assays is the ability to measure early indicators of cell stress and toxicity in liver cells. We report here a predictive, automated cell-based high-content imaging assay that simultaneously quantifies the multiplexed response from five different cellular properties in a single assay platform: cell loss, DNA content, intracellular reduced glutathione level, intracellular reactive oxygen species level and mitochondrial membrane potential changes. In this assay, liver cells (HepG2 cells, primary hepatocytes) were treated with a panel of compounds, including those known to be idiosyncratic human hepatotoxicants such as troglitazone, dantrolene, and tetracycline, as well as non-hepatotoxic compounds including rosiglitazone, aspirin, fluoxetine and melatonin. The cells were then labeled with specific fluorescent probes for the distinct cellular properties and automatically imaged and quantitatively analyzed using the Thermo Scientific ToxInsight IVT platform. By combining the individual responses from the five assay targets, we defined several multiplexed toxicity indices that accurately predicted drug-induced hepatotoxicity with greater than 80% specificity and sensitivity on the hepatic models tested. Additionally, the different multiplexed toxicity indices had better specificity and sensitivity in determining compound hepatotoxicity than measuring the individual assay target responses. We believe our approach shows an effective method of predicting hepatoxicity of compounds and thus reduce drug attrition.

PS 2541 GENE EXPRESSION PROFILING OF AN *IN VITRO* HUMAN SKIN MODEL AFTER PSORALEN PLUS ULTRAVIOLET LIGHT-INDUCED PHOTOTOXICITY.

L. F. Pratt[1], G. L. DeGeorge[1] and M. Cunningham[2]. *[1]MB Research Laboratories, Spinnerstown, PA and [2]Nanomics Biosciences, Inc., Cary, NC.*

To reduce the number of animals for safety screening of potential irritating chemicals and phototoxins, efforts have been made to develop more predictive in vitro assays. One model in more common use is reconstituted human epidermis (MatTek Epiderm) resembling in vivo human skin. In this investigation, these human skin models were exposed to a known skin irritant, 8-methoxypsoralen (8MOP), at a dose comparable to the EC10. The samples were also either kept in the dark or exposed to solar simulated light (SSL). Gene activity was analyzed with mRNA microarrays at 1, 6, and 20 hr to determine potential cellular and molecular mechanisms of action. Two levels of biological control samples were used: a) samples not treated with 8MOP and kept in the dark or exposed to UV light and b) samples treated with sodium dodecyl sulfate (SDS) [positive control]. Purified, labeled, and fractionated cRNA isolated from each of the biological samples were hybridized onto whole human genome mRNA expression microarrays, each containing 41,000 unique probes. Data analysis was done by a tiered approach. Coefficients of variation (CV) from all the probes passing quality measures or a total of 11,335 probes for each biological sample within the treatment groups ranged from 18.5-33.1%. The least variability was observed with the principal components analysis (PCA) for the negative control samples (those not exposed to 8MOP) and the samples exposed to 8MOP under dark conditions. The most activity was seen with 8MOP and SSL exposures at 6 and 20 hr as well as exposures to SDS, the positive control. Several genes in common between treatments with SDS and 8MOP were CXCL14, fibrillin2, tropomyosin alpha 1, CYP26B1, HSP70B AND VEGF-A. Funding was provided by NIEHS Grant No. 5-R44-ES-11927-02.

PS 2542 OXIDATIVE STRESS AND HYPOXIA AS FACTORS IN PHOTOTOXIC DAMAGE TO A RECONSTITUTED HUMAN SKIN MODEL: GENE EXPRESSION PROFILING EVIDENCE.

M. Cunningham[2], L. F. Pratt[1] and G. L. DeGeorge[1]. *[1]MB Research Laboratories, Spinnerstown, PA and [2]Nanomics Biosciences, Inc., Cary, NC.*

Currently there is worldwide emphasis on more predictive in vitro assays for phototoxicity, especially in screening dermatological and pharmaceutical products. Reconstituted human skin tissues modeling human skin in vivo were exposed to a known phototoxin, chlorpromazine (CPZ) with and without ultraviolet light, at a dose correlating with the EC10. The goal was to understand what cellular and genetic mechanism(s) contribute to the phototoxic response. The skin models were treated with CPZ in the dark as well as exposed to solar simulated light (SSL) and gene activity was measured with mRNA expression microarrays at 1, 6, and 20 hr post-exposure. For comparison, biological control tissues were concurrently treated with vehicle alone or left untreated CPZ, and kept either in the dark or exposed to UV light. Purified, labeled, and fractionated cRNA isolated from each of the tissue samples were hybridized onto whole human genome mRNA expression microarrays. The microarrays contained 41,000 unique probes corresponding to the full complement of sequenced human genes. The data was analyzed using a tiered approach. Coefficients of variation (CV) from all the probes passing quality measures or a total of 10,299 probes for each tissue sample within the treatment groups ranged from 5.3 to 19.2%. The least variability was observed with the principal components analysis (PCA) for the skin model samples not treated to CPZ under either dark or light conditions. The profiles for each treatment group were more similar by time point rather than by light/dark exposure. The numbers of down-regulated genes ranged from 10 to 52 and the numbers of up-regulated genes ranged from 96 to 236. Noteworthy genes which were down-regulated included genes involved in differentiation and normally expressed in epithelial tissue, such as CXCL14 and DAPL1. Several genes (e.g. HSPA6, ERO1L and ANGPTL4) related to oxidative stress and hypoxia were up-regulated. Funding was provided by NIEHS Grant No. 5-R44-ES-11927-02.

PS 2543 A NEW *IN VITRO* MODEL FOR IDENTIFYING LIVER SPECIFIC TOXICITY (LST).

J. M. McKim, B. Wallace, P. C. Wilga, C. Toole, H. Wagner, A. Swanson and K. Rutherford. *CeeTox, Inc., Kalamazoo, MI.*

In early drug discovery, the ability to rapidly identify compound liabilities while optimizing for desired drug qualities is critical for success in preclinical and clinical safety studies. Cell lines can provide information on general toxicity, but cannot identify organ specific toxicity. The aim of this study was to develop and optimize an *in vitro* system that could identify liver specific toxicity of highly toxic compounds and of compounds with low toxicity, often categorized as idiosyncratic. To accomplish this, a two cell model was used in this study, a rat hepatoma (H4IIE) cell line and rat primary hepatocytes grown in sandwich culture. Test compounds with known mechanisms of toxicity (rotenone, camptothecin, ANIT) were tested along with three groups of approved drugs, where one member of each group had shown after-market liver toxicity or a drug-drug interaction. The three groups evaluated included the antifungals (ketoconazole, itraconazole, fluconazole), the antidepressants (nefazodone, trazodone, buspirone), and the antidiabetics (troglitazone, pioglitazone, and rosiglitazone). The test compounds were applied to the culture

wells (0, 1, 5, 10, 25, 50, 100, and 300 μM) of either 96- or 24-well plates in replicates of 3 and allowed to incubate at 37°C with 5% CO_2 for 24 hr. Cell health was determined by membrane leakage, mitochondrial function, cell proliferation, GSH depletion, and caspase 3 activation. Liver specific markers for canalicular damage included GGT and AP. Inhibition of UGT1A1 and BSEP were determined in a cell-free system. Analysis of the data obtained yielded a toxicity profile that accurately identified ketoconazole, nefazodone, and troglitazone as the high risk compounds. In conclusion, the LST panel described provides an excellent method for assessing risk for liver specific toxicity.

PS 2544 DEVELOPING AN *IN VITRO* MODEL TO DETERMINE KIDNEY SPECIFIC TOXICITY (KST).

J. F. Pregenzer[1], K. Leach[2], B. Feng[3], B. Wallace[1], D. Keller[1], J. Willoughby[1] and J. M. McKim[1]. *[1]CeeTox, Inc., Kalamazoo, MI, [2]Compound Safety Prediction, Pfizer Global Research and Development, Groton, CT and [3]Pharmacokinetics and Drug Metabolism, Pfizer Global Research and Development, Groton, CT.*

Kidney toxicity is induced by a wide spectrum of marketed drugs, and renal toxicity is one of the major reasons for drug failure in early preclinical safety studies. Cellular models that can be used to predict compounds that may cause renal toxicity would be of great value. Most observed toxicity in the kidney occurs in the proximal tubule. Therefore, a human derived *in vitro* model that accurately reflects the in *vivo* orientation and function of the kidney would provide data specific to the function of kidney. Cryopreserved human renal proximal tubule cells (hRPTC) were obtained commercially and seeded onto 12-well transwell plates coated with collagen at a density of 100,000, 200,000 or 500,000 cells per well. The cells were allowed to grow for 7-10 days to establish tight junctions and proper polarity. Incubation with Lucifer Yellow demonstrated an adequate barrier between the apical and basolateral compartments. The presence of major renal drug transporters including OAT1, OAT3, and megalin was confirmed by either qRT-PCR or immunohistochemical staining. To further characterize transporter function, 5 μM of ^{14}C-PAH or 50 μM ^{14}C-TEA was added to the basolateral side of the cells. Transport of the radiolabeled compounds to the apical side suggested that these cells display functional OAT and OCT transport activity. OAT activity was further validated by probenecid inhibition. Incubation of these cells with the nephrotoxic compound cisplatin (1 to 500 μM) for 24 hr resulted in reduced cell viability, as measured by LDH release and propidium iodide staining. In contrast, cisplatin did not inhibit the growth of the kidney cell lines NRK-52E or LLC-PK1 at concentrations up to 500 μM. In conclusion, hRPTCs display kidney-specific functional activity and thus may be a sensitive model for predicting nephrotoxicity.

PS 2545 *IN VITRO* ASSESSMENT OF EYE TOLERANCE USING THE SKINETHIC HCE TEST METHOD APPLIED TO INGREDIENTS USED IN COSMETICS.

M. H. Grandidier, N. Alépée, S. Blond, J. Eilstein, D. Duché, J. Cotovio and J. R. Meunier. *L'Oréal, Aulnay sous Bois, France.* Sponsor: H. Toutain.

To comply with the EU Cosmetic Directive, accurate sensitive and predictive alternative methods are required in order to evaluate eye damage potential of chemicals. Linked to the complexity of both the eye irritation mechanism and the diversity of the intrinsic molecular behavior of chemicals, assessment of the potential eye irritation of ingredients is a complicated issue. The role of corneal epithelium is crucial because it represents the first line of defense against many types of injury. Thus we have used the standardized SkinEthic reconstructed human corneal epithelial (HCE) model to evaluate in vitro eye irritancy (Cotovio et al, 2010). We have determined the chemical reactivity parameter by measuring the depletion of synthetic peptide containing Cysteine or Lysine residues in contact with chemicals. The reactivity peptide binding assay (RPBA-HCE) defining the reactivity status of chemicals was optimized from the DPRA assay (skin sensitization evaluation) for assessing the eye irritancy using the SkinEthic HCE test method. The Prediction Model (PM), applied for a set of more than 180 chemicals, is based on a 5.95 % depletion cut off, with 2 categorizations (reactive and non reactive). In parallel, SkinEthic HCE protocol (10 min and 1 hr + 16 hrs post-exposure incubation) used with the same set of chemicals confirmed that the PM based on a 50 % viability cut-off allowed the drawing up of irritants and non irritants classes. Results (~1/3 reactive versus ~2/3 non reactive chemicals) showed that the measure of molecular reactivity of these chemicals used to orientate the tested chemicals towards the suitable time exposure allowed to discriminate irritants from non irritants. Consequently, the resulting performances of the testing battery combining SkinEthic HCE assays and chemical reactivity measurement greatly enhanced the applicability domain of the SkinEthic HCE assay.

PS 2546 USING *FUNDULUS HETEROCLITUS* TO STUDY BENZO(A)PYRENE EFFECTS ON THE HYPOTHALAMUS-PITUITARY-GONAD (HPG) AXIS.

F. T. Booc[1], C. Thornton[1], D. MacLatchy[2], A. Lister[2] and K. L. Willett[1]. *[1]Pharmacology, University of Mississippi, University, MS and [2]Biology, Wilfrid Laurier University, Waterloo, ON, Canada.*

Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BaP) alter aromatase (CYP19) expression. Because aromatase is responsible for converting androgens to estrogens, hormone concentrations in exposed individuals could also be affected. Altered steroid homeostasis may, in turn, negatively affect reproductive physiology and other downstream physiological processes. In order to further study possible physiological effects, a short-term reproductive bioassay was done using *Fundulus heteroclitus* adults exposed to waterborne concentrations of BaP (0, 1 or 10 μg/L) for 28 days. Males and females were kept separate days 0-14. The sexes were combined for days 14-28 so that spawning and reproductive success could be measured. BaP exposure did not alter male or female gonad somatic index (GSI) or liver somatic index (LSI) significantly. Similarly, sperm counts, egg production and fertilization success (n = 6 tanks per treatment; 3 fish of each sex per tank) were not altered by exposure. In conclusion, BaP-mediated changes in aromatase expression have the potential to cause changes in the HPG axis, but more endpoints will need to be analyzed (e.g. plasma steroid concentrations, LH and FSH expression, vitellogenin) before definitive statements can be made about its potential to negatively impact reproductive and developmental success at these concentrations. (Supported by NIEHS R03 ES018962)

PS 2547 PORCINE ADRENAL CORTEX MICROSOMES AS *IN VITRO* ALTERNATIVE ASSAY FOR SCREENING ON SEX STEROIDOGENESIS TOXICITY.

M. Roelofs[1, 2], M. van den Berg[1], A. H. Piersma[2, 1] and M. B. van Duursen[1]. *[1]Endocrine Toxicology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands and [2]National Institute for Public Health and the Environment, Bilthoven, Netherlands.*

The majority of experimental animal use in chemical risk assessment occurs in reproductive toxicity testing. Therefore, there is a high need for *in vitro* alternative assays. In this study we investigated the potential use of porcine adrenal cortex microsomes (PACMans) for screening of compounds for their potential interaction with the steroidogenic pathway. Porcine adrenals were obtained from the slaughterhouse and microsomal fractions were prepared through ultracentrifugation. PACMans were incubated with pregnenolone and NADPH whereupon production of dehydroepiandrosterone (DHEA), testosterone (T), and estradiol (E2) was determined using commercially available radioimmunoassays (RIAs). Our data show that PACMans contain all key enzymes of the steroidogenic pathway since DHEA, T, and E2 could all be determined. However, T and E2 production was very low at 8.53 and $2.3*10^{-3}$ pg/mg protein/min, respectively. These findings are supported by the low activity of aromatase (CYP19) in the PACMans (0.56 ± 0.06 pmol/hr/mg protein). In contrast, DHEA production could very well be determined (0.60 ng/mg protein/min) indicating a high cytochrome P450 17 (CYP17) activity. Adding CYP17 inhibitor SU10603 significantly reduced DHEA levels to background. Furthermore, concentration-dependent inhibition of DHEA production by ketoconazole (IC_{50} = 1 μM) and prochloraz (IC_{50} = 0.4 μM) but not bisphenol A was observed. This corroborates with findings in the H295R (human adrenocorticocarinoma cell line) assay. Although T and E2 production are too low for sensitive screening, PACMans could prove useful in testing for interactions with CYP17 activity. CYP17 is one of the key enzymes in sex steroidogenesis for which currently no specific rapid bioassay is available. Studies are ongoing to determine the predictivity and applicability of this assay, and will include screening of several potential endocrine disruptors.

PS 2548 EARLY DISRUPTION OF ANGIOGENESIS-RELATED GENES BY METHYL TERT-BUTYL ETHER (MTBE) LEADS TO VASCULAR SPECIFIC LESIONS IN THE ZEBRAFISH (*DANIO RERIO*).

J. A. Bonventre, L. A. White and K. R. Cooper. *Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ.*

MTBE disrupts normal vascular development in embryos. Exposure to 0.625 to 10mM MTBE resulted in a dose dependent increase in pooled blood in common cardinal vein, cranial hemorrhages and abnormal intersegmental vessels. Stage specific exposures were carried out to determine the developmental period most sensitive to MTBE vascular disruption. Embryos were treated with 10mM MTBE from

0-15, 0-24, 0-30 or 0-48 hours post fertilization (hpf) or 15-120, 24-120, 30-120 and 48-120hpf. Embryos treated until 15hpf or treated after 30hpf did not exhibit a significant increase in lesions, while embryos treated between 15-30hpf had a significant increase in MTBE specific vascular lesions ($p \leq 0.05$). We hypothesized that MTBE disrupts the regulation of genes in the vascular endothelial growth factor (VEGF) pathway. The effect of 5mM MTBE on VEGF associated genes was analyzed by qPCR at 24h, within the critical period. MTBE significantly ($p \leq 0.05$) decreased expression of VEGF-a and -c by 40 and 70% respectively, by had no effect on VEGF receptor-2 or HIF1-α. Expression of Wnt3a, VE-Cadherin5 and βactin were significantly decreased, while MMP-2 and -9 were not. To further understand the effect of MTBE on zebrafish development at time course analysis of global gene expression patterns was conducted using the Zebrafish Affymetrix GeneChip. Embryos were exposed to 6.25μM, 0.625mM or 5mM from 0-15, 0-24 or 0-30hpf. Preliminary evaluation of the data show the maximal number of genes with a greater than 2 fold-change occurred at 24hpf for all treatments. The least number of gene changes occurred at 30hpf. Taken together, these data suggest that vascular lesions observed with MTBE exposure develop as a result of disruption of genes expressed in the first 24h of development despite the fact that lesions are first apparent at 48hpf. These studies demonstrate critical periods in zebrafish vascular development and identify pathways sensitive to cardiovascular toxicants. Training Grant #ES07148 Center Grant #ES05022

PS 2549 EFFECT OF TRIAZINES ON TESTOSTERONE BIOSYNTHESIS IN BLTK1 MURINE LEYDIG TUMOR CELLS.

A. L. Forgacs[1], Q. Ding[1], I. T. Huhtaniemi[2], N. A. Rahman[2] and T. R. Zacharewski[1]. *[1]Michigan State University, East Lansing, MI and [2]University of Turku, Turku, Finland.*

Atrazine (ATR), Simazine (SIM), and Propazine (PPZ) are equipotent triazine herbicides that have been implicated in the etiology of testicular dysgenesis by altering steroidogenesis. To further investigate their effects on testosterone biosynthesis, ATR, SIM, and PPZ were examined in BLTK1 cells, a novel murine Leydig cell line (BLT-1 cells, clone K1) that possess an intact steroidogenic pathway producing low basal levels of testosterone (T), which can be induced by recombinant human chorionic gonadotropin (rhCG). ATR, SIM and PPZ (1, 3, 10, 30, 100, 300 and 600 μM) elicited dose-dependent increases in progesterone (P) and T levels up to 4-fold relative to DMSO at 24 hrs as determined by ELISA. Temporal analysis (300 μM at 1, 2, 4, 8, 12, 24 or 48 h) elicited comparable P and T profiles by all three triazines (ATR > PRO >> SIM) that were similar to the P and T profiles induced by rhCG. ATR elicited time- and dose-dependent induction of Star and Hsd17b3 mRNA levels while Hsd3b1, Cyp17a1 and Srd5a1 mRNA expression was repressed. PPZ elicited similar, albeit weaker, gene expression effects while SIM had minimal effects on steroidogenic gene expression. These results indicate that triazines alter P and T levels by regulating gene expression profiles of specific steroidogenic enzymes.

PS 2550 IMPROVEMENTS AND LIMITATIONS OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY TEST (BCOP, OECD TEST GUIDELINE 437) IN ROUTINE TESTING FOR SEVERE OCULAR IRRITANTS.

H. Raabe[3], A. Schrage[1], R. Curren[3], K. Norman[3], S. N. Kolle[2], M. Rey-Moreno[2], B. van Ravenzwaay[2] and R. Landsiedel[2]. *[1]Product Safety, BASF SE, Ludwigshafen, Germany, [2]Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany and [3]Institute for in vitro Sciences, Inc., Gaithersburg, MD.*

Data on eye irritation are generally needed for hazard identification of chemicals. Since the bovine corneal opacity and permeability (BCOP) test has been accepted by many regulatory agencies for the identification of corrosive and severe ocular irritants since September 2009 (OECD Test Guideline 437), we evaluated this alternative method using a broad variety of chemicals and formulations. Ten reference standards recommended in the TG437 and 21 substances (including historical false negatives [FN] and false positives [FP]) with published BCOP and *in vivo* data were tested in two labs (BASF and IIVS). Our results matched the published *in vitro* data very well, the concordance was 77% (24/31) with FN and FP rates of 20% (2/10) and 24% (5/21). However, one of the solid test substances, Dibenzoyl-L-tartaric acid, had a high variability in both labs resulting in false negative (FN) classifications in about half of the BCOP assays performed (n=8). Additionally as suggested by the guideline, we used histopathological evaluation of cross sections of treated corneas to identify false FN. This endpoint corrected the classification of some FN, but increased the number of false positives at the same time. Thus it did not increase of the overall predictivity. In parallel, we compared the opacitometer which was originally used in the development of the BCOP in the 1980s to a new opacitometer model with certified components, electronic data processing and calibration standards (glass filters). The data obtained with the new instrument correlated almost perfectly with the traditionally machine. Regardless of the utilized equipment, we recommend the use of calibration filters for stability, reproducibility and comparability of BCOP results from different laboratories.

PS 2551 ECVAM PREVALIDATION STUDY ON SKIN SENSITISATION ALTERNATIVES: UPDATE ON PROGRESS.

A. Angers-Loustau[1], D. Basketter[2], P. Aeby[3], S. Aiba[4], N. Alépée[8], T. Ashikaga[5], T. Cole[1], A. Compagnoni[1], G. Gerberick[6], S. Hoffmann[7], J. Ovigne[8], J. Richmond[9], H. Sakaguchi[10] and S. Casati[1]. *[1]European Commission (Joint Research Centre), Ispra, Italy, [2]DABMEB Consultancy Ltd., Sharnbrook, Bedfordshire, United Kingdom, [3]Colipa, Skin Tolerance Task Force, Brussels, Belgium, [4]Tohoku University Graduate School of Medicine, Sendai, Japan, [5]Shiseido Quality Assessment Center, Yokohama, Japan, [6]Procter & Gamble, Cincinnati, OH, [7]Seh Consulting & Services, Koln, Germany, [8]L'Oréal Research, Aulnay-sous-Bois, France, [9]Home Office, Dundee, United Kingdom and [10]Kao Corporation Safety Science Research Laboratories, Tochigi, Japan.*

Recent progress with in vitro assays in skin sensitization toxicology has resulted in the development of test methods which could make a valuable contribution to the replacement of the existing animal tests. ECVAM is currently evaluating three approaches: the Direct Peptide Reactivity Assay (DPRA), the Myeloid U937 Skin Sensitization Test (MUSST) and the human Cell Line Activation Test (h-CLAT) in a prevalidation study conducted in liaison with JaCVAM and ICCVAM. The main purpose of the study is to assess the robustness and reliability (within- and between-laboratory reproducibility) of the test methods. To this end, they are challenged with a set of 24 coded chemicals in three laboratories each. Results generated in this study will allow exploring the possible contribution of these tests to the prediction of skin sensitisers and their potency. An overview of the challenges and conclusions of the Transferability phase will be presented, as well as additional preliminary results from the testing phase with coded chemicals. In the case of the DPRA, the experimental part will be finalized and the results presented.
Disclaimer: The views expressed in this poster are purely those of the authors, and should not be regarded as an official position of the European Commission.

PS 2552 LEYDIG CELLS RESPONSES TO STIMULATION WITH LUTEINIZING HORMONE IN CULTURES FROM RATS OF DIFFERENT AGE.

P. Balbuena, J. L. Campbell, R. Clewell and H. J. Clewell. *The Hamner Institutes of Health Sciences, Research Triangle Park, NC.*

Leydig cells, which synthesize and secrete testosterone, are essential in the development and maintenance of male phenotype and reproductive capacity. Most of the information available in the literature on Leydig cells originated from evaluation of whole animals, intact testes, or tumor-derived cell lines. However, alterations in cellular function associated with immortalization limits the use of cell lines for studying normal function or chemical disruption of the Leydig cell itself. A viable system based on primary Leydig cell cultures would present a great advantage in elucidating mechanisms of action for compounds affecting Leydig cells steroidogenesis.
Primary cultures of Leydig cells were established from testes of 1, 3, and 5 month old Sprague Dawley rats. Passages of these cultures were performed until cells reached senescence. Cells were then tested for production of testosterone in response to Luteinizing hormone (LH) at 2, 4, 8, 16, and 24 hr. Since adult Leydig cells require LH stimulation to produce testosterone, LH induction and measurements of hormone production allowed us to assess variability due to time of exposure, passage of cells, and age of the primary cells in culture.
LH induced testosterone concentrations increased over time, with maximum levels 8-fold that of the control at 16 hr. for all ages. LH-induction of testosterone in the 1 and 3 months old rat cells reached 5-7 ng/ml, but in the 5 months old rat cells the levels were 0.05 ng/ml. Cells from 3 month old rats were viable up to 5 passages., although testosterone production decreased at passage 3. Passages of cells from 5 month old rats were not viable. This system shows considerable potential for the study of age dependent differences in normal Leydig cell function without the additional complications inherent in an in vivo system. Furthermore, the ability to maintain testosterone production in passaged cells presents an opportunity for long-term study of molecular mechanisms of compounds associated with Leydig cell abnormal function.

PS 2553 AN ORGANOTYPIC MICROLIVER PLATFORM FOR HIGH-THROUGHPUT DRUG TESTING.

S. Messner[1], B. Machi[1], S. Hammad[2], J. M. Kelm[1], J. G. Hengstler[2] and W. Moritz[1]. [1]*InSphero AG, Zürich, Zürich, Switzerland and* [2]*IfADo Dortmund, Technical University of Dortmund, Dortmund, Germany.* Sponsor: C. Corton.

Predictive toxicology testing is a major challenge in regulatory toxicology and drug discovery. To avoid extensive use of animal experiments, primary hepatocytes are commonly used to study drug metabolism, enzyme induction and compound hepatoxicity in vitro. However, in monolayer cultures, dedifferentiation processes lead to a severe loss of liver-specific functions. While 2D sandwich cultures improve maintenance of hepatocyte function, these systems are technically challenging, of little use for chronic or idiosyncratic toxicity testing and only fairly suited for higher throughput. In order to preserve liver-specific functions of hepatocytes over extended time periods and to provide a versatile platform for toxicology testing, we developed a novel scaffold-free, organotypic production technology for a 3-dimensional culture of hepatic microtissues in a standard 96-well microtiter plate format. This format enables regular medium exchange and compound supplementation in hanging drops. Microtissues from primary rat or human hepatocytes are produced with the new GravityPlus production technology by top- loading of a cell suspension at a density of 2000 hepatocytes per well. Inside the hanging drop, rapid microtissue formation takes place within 3-4 days. The GravityPlus system allows the production of regular-sized microtissues with a morphology and architecture close to native liver tissue, in particular when co-cultured with non-parenchymal liver cells. The metabolic activity and liver-specific parameters (e.g. CYP450 induction, albumin and urea secretion) are consistently superior to hepatocyte sandwich culture, especially at culture periods of more than 7 days. Our results demonstrate the validity and suitability of the GravityPlus hanging drop platform for liver microtissue culture, offering a highly functional and reliable in vitro toxicology testing system which can easily be implemented in standard lab automation processes.

PS 2554 QUANTIFICATION OF UVA-INDUCED REACTIVE OXYGEN SPECIES IN PRIMARY HUMAN KERATINOCYTES TREATED WITH ANTIOXIDANTS.

K. Norman, H. Inglis and G. Mun. *Institute for in vitro Sciences, Gaithersburg, MD.* Sponsor: E. Dahl.

Environmental exposure of the skin to ultraviolet radiation makes it a primary target for UVA-generated reactive oxygen species (ROS). An overabundance of ROS causes oxidative stress which may result in the oxidation of proteins, lipids, and nucleic acids. This oxidative damage is a major contributor to photoaging and is associated with skin carcinogenesis. In order to mitigate the damaging effects of ROS, antioxidants are increasingly being added to formulations of topical products. The goal of this study was to develop an in vitro method capable of evaluating the antioxidant potential of ingredients and formulations in primary human keratinocytes (NHEKs). NHEK cells were seeded in 96-well plates and incubated with the fluorescent ROS-detecting probe, 5 (and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA), for 40 minutes, then exposed to a serial dilution of antioxidants/antioxidant-containing formulations for 1 hour. ROS were generated by exposing the cultures to UVA light for 50 minutes (1.6-1.8 mW/cm2), and then detected by fluorescence measurements (ex/em 485/530 nm) of the cells. Cytotoxicity was assessed concurrently using the neutral red uptake assay. Using this method, we evaluated several known antioxidants and antioxidant-containing formulations for their ROS-reducing capabilities. Based on our results, several antioxidants significantly reduced ROS levels compared to untreated controls. Other antioxidants which did not show this reduction were likely not water soluble and/or poorly bioavailable to cells. Overall, our results indicate that this method may provide a valuable in vitro tool for assessing antioxidant capacity in a biologically relevant model.

PS 2555 *IN VITRO* ASSESSMENT OF SKIN IRRITATION POTENTIAL OF SURFACTANT BASED FORMULATIONS USING 3-D SKIN RECONSTRUCTED TISSUES AND CYTOKINE EXPRESSION ANALYSIS.

L. Gandolfi[1], N. Tierney[1], D. Johnson[1], R. Walters[1], M. Fevola[1], E. Gunn[1], K. Martin[1], A. Kong[2], A. Hilberer[2], N. Barnes[2], N. Wilt[2], J. R. Nash[2], H. Inglis[2], H. Raabe[2] and G. Costin[2]. [1]*Johnson & Johnson, Skillman, NJ and* [2]*Institute for in vitro Sciences, Inc., Gaithersburg, MD.*

A goal of personal care products manufacturers is to develop increasingly milder formulations. To this end, reproducible *in vitro* systems can accurately assess the irritation potential of the products, thus avoiding the use of animals for testing. The three-dimensional EpiDerm™ model (MatTek Corp.) provides a testing platform for Johnson & Johnson's skin irritation assessment program targeting raw ingredients and final formulations. The testing program we have developed evaluated the potential dermal irritation of over 150 amphoteric and/or anionic surfactant-containing candidate formulations or individual raw ingredients. The formulations were diluted to 10% in water and were applied onto the surface of the 3-D tissues for 1 h, followed by 24 h post-exposure for cytokine expression. The potential dermal irritation of the candidates was evaluated by MTT viability and IL-1α release. Another goal of the program was to qualify two representative benchmark materials with known skin irritation potential for use as references for the skin irritation evaluation of formulations with new surfactant ingredients. We have developed a database and established a range of irritation responses of the benchmarks using the IL-1α endpoint. Comparison of the potential dermal irritation of new formulations to existing mild formulations guides formulation development for new mild cleansing products. Most recently, we have demonstrated the reliability of the test system for the assessment of irritation potential of final formulations. We are currently expanding our database and work towards establishing a correlation between the *in vitro* pre-screening approach and clinical testing. This testing platform integrates the efforts made to meet the mildness testing needs of global manufacturers of personal care products that focus on developing increasingly milder lines of formulations to be applied to the skin.

PS 2556 USE OF EPIDERM FULL THICKNESS (EFT) SKIN CULTURES AS AN *IN VITRO* MODEL FOR WOUND HEALING.

P. Hayden[2], M. Sachdeva[1], P. P. Shah[1], U. S. Desai[1], R. Patlolla[1] and M. Klausner[2]. [1]*Pharmacy, Florida A&M University, Tallahassee, FL and* [2]*MatTek Corporation, Ashland, MA.*

Background: Wound healing is a natural process which involves the regeneration of epidermal and dermal tissue with expression of growth factors and inflammatory markers. The objective of this study was to evaluate the EpiDerm full thickness (EpiDerm-FT, EFT) skin culture as an in-vitro wound healing model to understand the healing process after chemical induced wounding.
Methods: A strong base, 32N KOH and 2 strong acids, conc H2SO4 and acrylic acid, were selected for making chemical wounds. The dose of chemical and exposure time required to generate a significant wound were optimized. After inducing a wound, cultures were washed thoroughly with PBS and incubated at 37°C for 6 days. The EFT tissues were collected for histological, immunohistochemistry and western blot studies. The culture medium was collected to study the release of inflammatory markers such as Interleukin-6 (IL-6) and Interleukin-8 (IL-8).
Results: Hematoxylin and eosin staining showed significant damage to the stratum corneum and upper layers of the epidermis for 32N KOH, deeper epidermal damage for conc H2SO4 and dermal damage for acrylic acid. Western blot studies showed that the ratio of collagen IV to β-actin was increased in the order of acrylic acid > conc H2SO4 > 32N KOH while the ratio of KI67 to β-actin was increased in the order of conc H2SO4 > acrylic acid > 32N KOH. Similar results were observed with immunohistochemistry studies suggesting a strong correlation between the two techniques. These data were further supported by cytokine studies which showed that the release of IL-6 and IL-8 was significantly increased ($p<0.001$ vs control) on day 1 and thereafter it was significantly decreased ($p<0.01$ vs control) for all chemicals suggesting wound healing.
Conclusion: These results strongly support that the EFT cultures could be used as an in-vitro model for studying the cutaneous wound healing process.

PS 2557 *IN VITRO* BILIARY CLEARANCE OF PERFLUOROOCTANOATE IN SANDWICH-CULTURED HEPATOCYTES FROM RATS.

D. Nabb, R. Mingoia and X. Han. *DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE.*

Rat hepatocytes regain polarity and maintain expression levels of uptake and efflux transport proteins for several days if cultured in a sandwich configuration, and therefore, are an ideal system for the evaluation of biliary excretion of xenobiotics in vitro. In vivo biliary clearance of xenobiotics can then be estimated from the in vitro-determined parameters. In the present study, the biliary excretion of [3H]-taurocholate (served as positive control) and [14C]-perfluorooctanoate (PFO) in 96-h sandwich-cultured male rat hepatocytes was determined by differential cumulative uptake of substrate in the monolayers preincubated in standard buffer (intact bile canaliculi) and in Ca2+-free buffer (disrupted bile canaliculi). Preliminary results show that biliary excretion index (the percentage of biliary excretion relative to the cumulative uptake of a substrate) of taurocholate and PFO was 40% and 18%, respectively. Future experiments will be conducted in female rat hepatocytes and in human hepatocytes to understand the sex- and species-dependence of biliary excretion potential of PFO.

PS 2558 IDENTIFICATION OF NOVEL BIOMARKERS OF FORMALDEHYDE TOXICITY IN HUMANS USING FUNCTIONAL TOXICOGENOMICS IN YEAST.

M. North[1], C. Romero[1, 2], A. Loguinov[1], M. T. Smith[2], L. Zhang[2] and C. D. Vulpe[1]. [1]*Department of Nutritional Science & Toxicology, The University of California Berkeley, Berkeley, CA and* [2]*Division of Environmental Health Sciences, School of Public Health, The University of California Berkeley, Berkeley, CA.*

Formaldehyde is a widely used, economically important chemical. There is extensive occupational and environmental exposure to formaldehyde all over the world, as it is released from household products, and is a component of tobacco smoke. It is an established human carcinogen that causes nasopharyngeal cancer, and there is an association between leukemia and occupational exposure to formaldehyde. At present the mechanistic basis of the hematotoxicity of formaldehyde is unclear. We have used a functional toxicogenomics approach to identify the genes that modulate the cellular toxicity of formaldehyde in the model eukaryote *Saccharomyces cerevisiae*. *S. cerevisiae* has many tools available for genetic study, including a complete set of non-essential gene deletions, genetically tagged so that individual strains can be identified in competitive growth experiments. Using this set, parallel systematic analysis can be completed to identify mutant strains that are susceptible to toxicant treatments. We have used this technique to determine the cellular processes targeted by formaldehyde. Mutants deficient in the repair of DNA strand breaks were identified as having increased sensitivity, indicating that formaldehyde causes DNA damage. Several proteins involved in mRNA processing, including the Ski and PAN complexes are required for formaldehyde tolerance. Strains lacking components in the Golgi transport complex and the ER-Golgi intermediate compartment are also sensitive, implicating intracellular trafficking as important. Several of the genes identified have human orthologs with conserved biological function, supporting the notion that the mechanisms of toxicity identified are relevant to human disease. This study highlights *S. cerevisiae* as a simple but valuable model for identifying biomarkers for genetic susceptibility to toxicant-related disease.

PS 2559 VALIDATION RESULTS AND METHODS FOR IMPROVING *IN VITRO* TIER 1 ENDOCRINE DISRUPTION SCREENING ASSAYS.

C. Toole, D. P. Blakeman, N. Nichols, M. Clark, H. Wagner, B. Wallace and J. M. McKim. *CeeTox, Inc., Kalamazoo, MI.*

The Endocrine Disruption Screening Program (EDSP) was initiated by the Environmental Protection Agency (EPA) in order to develop a screening program to evaluate whether or not certain chemicals could have hormonal effects in humans. As a result Tier 1 assays, *in vitro* and *in vivo* were selected to evaluate endocrine, androgen and thyroid effects of commercial chemicals and environmental contaminants. CeeTox has established and run reference compounds outlined by the EPA for these five *in vitro* Tier 1 assays (Steroidogenesis, Aromatase, ER and AR Binding, and ER Transactivation) according to OECD guidelines and EPA protocols. Proficiency data was generated from reference compounds run in these Tier 1 assays using modifications that improve the assays by reducing false negative and false positive results and by identifying weak areas in the established protocols. As an example, for the ER transactivation assay, utilizing of the Hela-9903 cell line, inclusion of solubility and cytotoxicity assessments assist in data interpretation. Additional controls were incorporated in order to reduce misinterpretation of the data. These included modification of the plate format to decrease the potential for edge effects and incubation with a strong antagonist (ICI 182,780) to determine background luminescent values.

PS 2560 A NOVEL STRATEGY FOR *IN VITRO* ASSAY PLATFORM TO PREDICT SKIN SENSITIZING POTENTIAL OF BROAD RAW MATERIALS.

M. Miyazawa[1], Y. Nukada[1], K. Saito[1], H. Sakaguchi[1], N. Nishiyama[1] and F. Gerberick[2]. [1]*Kao Corporation, Tochigi, Japan and* [2]*Procter & Gamble Company, Cincinnati, OH.*

Recent changes in regulatory restrictions and social views against animal testing necessitated the development of reliable in vitro tests for predicting skin sensitizing potential of a broad range of industrial chemicals. To this end, we propose a novel strategy for assessing sensitization using an in vitro platform against a variety of raw materials, taking into account the applicability and limitations for each assay.
Direct peptide reactivity assay (DPRA), based on the covalent binding between proteins and haptens, and human cell line activation test (h-CLAT), emulating dendritic cell activation in the skin during sensitization, have been currently prevalidated under ECVAM. Accordingly, we developed a battery system by combining DPRA and h-CLAT with the in silico program, DEREK. This optimized integrated testing strategy demonstrated higher sensitivity (89%) and specificity (80%) than either DPRA, h-CLAT or DEREK alone: indicating the reliability of the battery approach against highly-purified and single chemical substances. However, there is no available in vitro assay for complex mixtures and lipophilic chemicals. Thus, we further developed a HTP-compatible assay to measure the production of reactive oxygen species (ROS). The resulting cell-based ROS assay not only demonstrated relatively high accuracy (83%) with LLNA, but also predicted the sensitizing potential of several water-soluble mixtures. Furthermore, we determined gene expression profiles in EpiDerm after application of a known allergen DNFB. A unique set of genes encoding redox regulatory enzymes was upregulated significantly by DNFB, but not by a non-allergen, benzalkonium chloride. The selected target gene expression might enable us to predict the sensitizing potential of highly-lipophilic chemicals, since test samples are applied to EpiDerm in the same way as animal testing. Our use of various methods is an attempt to meet the challenge of finding a battery of in vitro assays that can evaluate a broad range of raw materials.

PS 2561 GENE EXPRESSION ANALYSIS OF PRECISION-CUT HUMAN LIVER SLICES INDICATE STABLE EXPRESSION OF HEPATOXICITY RELATED GENES.

M. G. Elferink[2], P. Olinga[1], E. M. van Leeuwen[3], S. Bauerschmidt[3], J. Polman[3], W. G. Schoonen[4], S. H. Heisterkamp[5, 6] and G. M. Groothuis[2]. [1]*Pharmaceutical Technology and Biopharmacy, Groningen Research Institute for Pharmacy, Groningen, Netherlands,* [2]*Pharmacokinetics, Toxicology and Targeting, Groningen Research Institute for Pharmacy, Groningen, Netherlands,* [3]*Molecular Design and Informatics, MSD, Oss, Netherlands,* [4]*Toxicology &Drug Disposition, MSD, Oss, Netherlands,* [5]*Biostatistics and Research Decision Sciences, MSD, Oss, Netherlands and* [6]*Bioinformatics Centre, University of Groningen, Groningen, Netherlands.* Sponsor: A. Vickers.

In the process of drug development it is of high importance to test the safety of new drugs with predictive value for human toxicity. A promising approach of toxicity testing is based on changes in the gene expression profile of the liver. Toxicity screening based on animal liver cells cannot be directly extrapolated to humans due to species differences. For the prediction of human specific toxicity, we evaluated precision-cut human liver slices as in vitro model. The liver slices contain all cell types of the liver in their natural architecture. This is important since drug-induced toxicity often is a multi-cellular process, involving not only hepatocytes but also Kupffer, stellate and endothelial cells. Previously we showed that toxicogenomic analysis of rat liver slices is highly predictive for rat in vivo toxicity. In this study we investigated the levels of gene expression during incubation up to 24 hours with Affymetrix microarray technology in 10 different human livers. The analysis was mainly focused on genes related to stress and toxicity, phase-I and phase-II metabolizing enzymes and drug transporters. From the total amount of probe sets analyzed (54675) the expression of 3% changed significantly during incubation (FC>2, $p<0.05$). Pathway mapping showed that these differentially expressed genes were significantly associated with cytoskeleton remodeling, extracellular matrix and cell adhesion In the categories stress and toxicity, drug transporters and metabolic enzymes, changes were minimal. These results indicate that precision-cut human liver slices represent a valuable model for human in vitro hepatotoxicity testing.

PS 2562 MEDIA SERUM LEVELS AND *IN VITRO* HEPATIC ABSORPTION OF LINDANE.

E. L. Croom and R. A. Pegram. *Office of Research and Development, National Health and Environmental Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC.*

High plasma protein binding is known to reduce the tissue uptake of chemicals in vivo, but the extent of its importance in vitro is less clear. Experiments were conducted to determine the cellular uptake of lindane in vitro under different conditions. Lindane was selected because of its high plasma protein binding, ~90%, and low limit of detection. In vitro assay conditions commonly range from 0 to 20% serum but sometimes involve media containing 75% to 100% serum. In vitro dosing schedules for viable cells often extend to 24hrs before the media is changed. Rat hepatocytes in 12 well plates were exposed to 1μM lindane. Media was supplemented with fetal calf serum (FCS). FCS levels were varied and media contained 0%, 5%, 20%, 50%, 75% or 100% FCS. After incubation, lindane was hexane extracted from tissue and media. At 24 hrs the highest concentrations of lindane were found in hepatocytes cultured in 100% FCS, while the lowest concentrations were found in hepatocytes cultured in serum-free media. In wells containing the lowest serum concentrations, very little lindane was recovered from the media or cells, suggesting the unbound lindane was absorbed and metabolized. Human liver slices (20 mg) were used under similar conditions but in the absence of a regenerating system to limit the loss of lindane through metabolism. At 5 minutes, most of the lindane was present in the liver slices, with the highest concentrations found in tissue in

serum-free media. At 24 hrs, the greatest concentrations of lindane were in tissues with low serum concentrations and the lowest levels of lindane were found in tissues in 100% FCS. Taken together, these results suggest that at low chemical concentrations high serum levels can delay the entry of lindane into tissue in vitro. In systems with high metabolic capacity this limited availability would likely extend the in vitro half-life of a compound. (This abstract does not necessarily reflect U.S. EPA policy).

PS 2563 AN *IN VITRO* MODEL FOR THE STUDY OF BACTERIAL COLONIZATION OF HUMAN EXTRAPLACENTAL MEMBRANES: ROLE OF ANTIMICROBIAL PEPTIDES.

E. Boldenow[1], S. Jones[1], C. Xi[1], R. Lieberman[2] and R. Loch-Caruso[1]. [1]*Environmental Health Sciences, University of Michigan, Ann Arbor, MI and* [2]*Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI.*

Preterm birth is a widely recognized, costly public health problem and is the leading cause of infant mortality. Though the etiology of preterm birth is not fully understood, preterm birth is associated with infection of the gestational compartment. We developed an in vitro system for the coculture of bacteria and human extraplacental gestational membranes, with the aim of using this system to study toxicant modification of microbial infection of the membranes. We used Streptococcus agalactiae (GBS) because this bacterium is commonly found in the vaginal tract and untreated GBS infection has been associated with preterm birth. Gestational membranes were affixed to Transwell frames (without synthetic membranes) in such a manner that the full thickness gestational membranes provided a tissue barrier between the Transwell culture compartments. The GBS was added to the decidual side of the membranes in the Transwell culture, and the tissues were cocultured for 4, 8 and 24 hours with GBS. GBS colony forming units were determined for both the media and the homogenized tissue. Recovered GBS colony counts decreased over time suggesting that a robust innate immune response remained active in culture. Antimicrobial peptide expression was identified in the gestational membranes using immunohistochemistry (IHC). Human beta-defensin (HBD)-2 expression, but not HBD-1, HBD-3, HBD-5, cathelicidin or elefin, was greater in the amnion of GBS-treated membranes compared to controls, suggesting that HBD-2 expression in the gestational membranes may be responsible for the killing of GBS over time. This in vitro system has potential applications for future studies of toxicant influences on bacterial infection of gestational membranes. Moreover, our results suggest that toxicant inhibition of the antimicrobial peptide HBD-2 deserves further study as a possible mechanism by which toxicants could increase susceptibility to gestational compartment infection.

PS 2564 EVALUATION OF AN ORAL CARE PRODUCT SAFETY SCREENING PROGRAM UTILIZING THE *IN VITRO* SKINETHIC HUMAN GINGIVAL EPITHELIUM (RHG) AND ORAL BUCCAL (RHO) MODELS.

L. Wurzburger[1], P. Kazmi[1], T. Re[1], A. Alonso[2], B. Bertino[2], N. Barnes[3], A. de Brugerolle de Fraissinette[2], A. Hilberer[3], H. Raabe[3], N. Wilt[3] and V. Srinivasan[1]. [1]*L'Oreal USA Products, Clark, NJ,* [2]*SkinEthic Laboratories, Nice, France and* [3]*Institute for in vitro Sciences, Inc., Gaithersburg, MD.*

Assuring the safety of personal care products without testing in animals is a goal common to many personal care products manufacturers, due to both ethical concerns for animal welfare, as well as the limited relevancy of animal models to predict human responses. Towards this goal, the cosmetics and personal care industry has increasingly relied upon human cell-based 3–dimensional reconstructed tissues to evaluate the safety of their product candidates in various target tissues. Accordingly, we have developed a program for screening the potential irritancy of teeth whitening products in oral mucosal tissues using commercially-available oral buccal (SkinEthic RHO) and gingival (SkinEthic RHG) models. Four formulations containing H_2O_2 at various concentrations and one sodium bicarbonate were tested at four exposure times to determine ET_{50} values. Three irritancy endpoints were measured after each exposure: viability using the MTT conversion assay, the amount of IL-1α released from the tissues, and histological changes. Both models presented the same rank order of the five materials, with increases in the ET_{50} values correlating with decreases in H_2O_2 concentration. The formula containing sodium bicarbonate, however, was non-toxic in both models. Histological analysis confirmed the MTT results and provided evidence of the chemical impact upon cellular and tissue morphology. IL-1α release did not appear to be as sensitive as the MTT assay at shorter exposure times, although it may be useful to differentiate among formulations predicted by the MTT assay to be of low irritation potential. Our results suggest that the MTT viability and histology endpoints in 3–D human oral reconstructed tissues can provide useful predictive information to support an oral care products safety program.

PS 2565 TRANSLATIONAL RESPONSE TO ARSENITE LEADS TO P-BODY AND PAB1P-CONTAINING GRANULE ASSEMBLY IN THE YEAST *SACCHAROMYCES CEREVISIAE.*

V. Stribinskis, M. W. Gordon, J. P. Moore, S. R. Ellis and K. S. Ramos. *Biochemistry and Molecular Biology, University of Louisville, Louisville, KY.*

Environmental stress of eukaryotic cells rapidly inhibits translation initiation and causes shuttling of a subset of cytoplasmic mRNAs and proteins away from the translational machinery into foci termed cytoplasmic processing bodies (PBs) and stress granules (SGs). These cytoplasmic aggregates contain different subset of proteins and often associate with each other, suggesting dynamic linkages between mRNA storage and decay. In mammalian cells, cytoplasmic mRNAs redistribute during arsenic stress to both PBs and SGs; however, the mechanisms and signaling pathways that reprogram mRNA translation and decay remain obscure. Yeast has been successfully used to unveil networks of transcriptional reprogramming of gene expression in response to arsenite. Here we demonstrate that arsenite transiently inhibits translation initiation at doses that affect neither cell viability nor growth. This inhibition leads to rapid induction of PBs and Pab1p-containing granules (mRNA poly(A)-binding protein). In the context of signaling, we show that arsenite induces rapid phosphorylation of eIF2α, which requires Gcn2 kinase. All aforementioned events are not completely abrogated, but rather delayed in cells lacking Gcn2 kinase, suggesting that phospho-eIF2α-independent mechanisms of translational control are also induced by arsenite. In contrast to the observed colocalization of translation initiation factors eIF4E and eIF4G with Pab1p in yeast SGs during glucose depletion, eIF4E and eIF4G were absent from Pab1p-containing granules upon arsenite stress, suggesting that mRNA relocalization to cytoplasmic granules can occur without factors that promote closed loop mRNP complexes. Our observations share similarities with those in mammalian systems, thus suggesting that the power of yeast genetics provides a good model to study how cells regulate cytoplasmic mRNAs in response to arsenite stress.

PS 2566 VALIDATION OF *IN VITRO* DIGESTION MODELS FOR POLYCYCLIC AROMATIC HYDROCARBON BIOACCESSIBILITY FROM SOIL USING THE *IN VIVO* SWINE MODEL.

K. James[1], R. E. Peters[1], B. Laird[1], W. Ma[2], M. Wickstrom[3], G. Stephenson[2] and S. D. Siciliano[4]. [1]*Soil Science, University of Saskatchewan, Saskatoon, SK, Canada,* [2]*Stantec Consulting Inc., Guelph, ON, Canada,* [3]*Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada and* [4]*Toxicology Group, University of Saskatchewan, Saskatoon, SK, Canada.* Sponsor: L. Weber.

Polycyclic aromatic hydrocarbons (PAHs) are a class of lipophilic organic contaminants commonly found in soil. Particular PAH compounds have been identified as known carcinogens. The total carcinogenic potential of PAHs can be expressed as the total potency equivalency factor (PEF), which is based on the relative potency of each compound to benzo(a)pyrene. PAH exposure from soil primary occurs through incidental ingestion and in-vitro digestion models, such as the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) and Relative Bioaccessibility Leaching Procedure (RBALP), can be used to estimate the bioaccessibility of ingested environmental contaminants. Bioaccessibility estimates from in-vitro models need to be validated using an in-vivo model for use in Human Health Risk Assessment (HHRA). The chemical properties of PAHs indicate that they will preferentially partition to lipophilic phases, therefore in-vitro models may require the addition of a lipophilic membrane to act as a lipid sink for PAH absorption. Using eight PAH contaminated soils we compare the release of the total PEFs from the SHIME and RBALP with and without the addition of a lipophilic membrane and then compare these results with the PEFs released from the swine model. For both models, the SHIME and RBALP, the addition of a lipophilic membrane significantly ($p < 0.05$) increases the release of PEFs from soil. However, the release of PEFs from the SHIME model with the addition of a lipophilic membrane is the only model that correlates to the PEFs released from the swine model. The amount of PEFs released in the swine model shows a relationship with the individual soil fugacity capacity, indicating that individual soil properties influence the release of PEFs.

PS 2567 *IN VITRO* EVALUATION OF AIRWAY TOXICITY USING THE EPIAIRWAY ORGANOTYPIC *IN VITRO* HUMAN AIRWAY MODEL.

G. R. Jackson, J. Bolmarcich, H. Kandarova, S. Letasiova, M. Klausner and P. J. Hayden. *MatTek Corp., Ashland, MA.*

Recent REACH legislation has heightened the need for validated in vitro airway toxicity models. However, in vitro determination of airway toxicity potential is problematic since submerged monolayer cell cultures are not amenable to dosing of

vapors or water insoluble chemicals. The EpiAirway model is an organotypic air-liquid interface culture produced from normal human airway epithelial cells. EpiAirway more adequately reproduces in vivo airway epithelial morphology and is more amenable to dosing since the apical surface is exposed to the atmosphere. Here we describe an approach to validate the EpiAirway model for airway toxicology applications. Two dosing protocols were developed and evaluated: 1) Direct application of test chemicals in aqueous or corn oil vehicles, and 2) a vapor cup method for dosing volatile chemicals. Dose response experiments were conducted to determine EC50 concentrations for loss of culture viability, transepithelial electrical resistance or release of inflammatory mediators. EC50 concentrations were correlated with OSHA established Immediately Dangerous to Life and Health (IDLH) concentrations. Direct application was found to perform well for chemicals with IDLH values up to ~1,000 mg/m3. A prediction model based on EC50 (culture viability) after direct application was established for 15 chemicals. A correlation coefficient of r2=0.79 was obtained for the entire chemical set. Alkyl amines were found to be over-predicted by the direct application protocol. With alkyl amines removed from the data set the correlation improved to r2=0.98. Direct application of chemicals with IDLH values above ~1,000 mg/m3 was generally not possible. A vapor cup dosing protocol was developed for these chemicals. To-date vapor cup results for 11 volatile chemicals including a diverse range of chemical classes produced a correlation coefficient of r2 = 0.83 with respect to in vivo IDLH values. Thus, the prediction models appear to provide promise for in vitro determination of airway toxicity. Future work will validate these models with larger chemical sets.

PS 2568 DEVELOPMENT OF THE REPLACEMENT OCULAR BATTERY-ROBATT-TIERED TESTING STRATEGY OF ALTERNATIVE TOXICOLOGY TESTS TO REPLACE THE NEED FOR RABBIT EYE TESTS.

D. Cerven, G. DeGeorge and M. Piehl. *MB Research Laboratories, Spinnerstown, PA.*

Using a series of non-animal assays in a tiered approach, the Replacement Ocular Battery (ROBatt) accurately predicts the categories of acute ocular irritation corresponding to OECD, EPA and GHS guidelines. At present, no single alternative assay has been accepted by regulatory agencies to completely replace the use of live animals. The Bovine Cornea Opacity/Permeability test (BCOP) has been accepted by OECD as a screen for severe and corrosive materials. The Cytosensor Microphysiometer has been accepted for sub-severe testing but is applicable only for aqueous-based materials. The ROBatt approach uses a series of two to three non-animal assays to categorize both aqueous and non-aqueous materials. An NIH/FDA Grant has been awarded to MB for development the ROBatt decision tree criteria. Initial screening will use the Chorioallantoic Membrane Vascular Assay (CAMVA) to discriminate slight or non-irritants from moderate to severe irritants. Slight or non-irritating materials will be categorized using the Porcine Cornea Confocal Assay (PorFocal). The BCOP will be used for discriminating between moderate and severe to corrosive materials and the Porcine Cornea Opacity Reversibility Assay (PorCORA) will be used to categorize severe irritants and corrosives. Fifty validation chemicals from the ECETOC database of ocular irritation will be used initially. Having performed over 6,700 CAMVA, 5,700 BCOPs, and nearly 100 PorCORA assays, the researchers are confident of the ability to completely categorize any material to international standards.

PS 2569 THE EYES HAVE IT: CALF *VERSUS* ADULT EYES IN THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY.

D. A. Donahue[2], D. Cerven[1], G. DeGeorge[1] and J. Avalos[2]. [1]*MB Research Laboratories, Spinnerstown, PA and* [2]*Kao Brands Company, Cincinnati, OH.*

The Bovine Corneal Opacity and Permeability (BCOP) assay uses excised bovine corneas obtained from cattle slaughtered for food use. Consequently, the age of the cattle is generally not provided with the corneas. This introduces a variable at the start of the assay, which can be reduced by using eyes from cattle at a defined age. The OECD guidelines for the testing of Chemicals No. 437 recommends the use of eyes from cattle 6 to 12 months of age but encourages investigators to report the estimated age and/or weight of the animals providing the corneas. In a commercial operation, capable of killing large numbers of cattle to provide suitable numbers of eyes, it may not be possible to determine age and weight since animals are received from a number of suppliers. Additionally, random-source animals typically have not been raised in constant environment and are subject to numerous environmental variables. This study looked at the effect of the cattle's age on the BCOP assay. The calf eyes used in this study were obtained from a well-managed barn-raised herd, which had weekly veterinary monitoring and controlled feed and medication. In this study we compared the In Vitro Scores (IVS) from random source cattle corneas with those of corneas from a well-managed calf herd of 4 to 5 months of age. Thirty over the counter cleaners, hair dyes, hair sprays, deodorants and moisturizers, which had IVS scores obtained from random-age animals were used in the evaluation. The IVS scores from the random aged animals ranged from -0.62 to 111.58. The IVS scores from age-defined calf eye corneas ranged from -1.11 to 22.86.

PS 2570 APPLICATION OF AVAILABLE ALTERNATIVE METHODS TO PREDICT SKIN SENSITIZATION OF PRESERVATIVES.

S. Martinozzi Teissier, C. Piroird, S. Ringeissen, J. Eilstein, D. Duché, J. Ovigne and J. Meunier. *L'Oréal, Aulnay sous Bois, France.* Sponsor: H. Toutain.

Contact sensitizers are reactive molecules that modify skin proteins to form antigen recognized by specific T cells. In addition to the haptenation mechanism, contact sensitizers induce several phenotypic and functional changes of dendritic cells that allow them to migrate to the lymph node, present antigen and prime efficiently hapten-specific T cells. Risk assessment of chemicals for skin sensitization so far relies on animal assays. Concerned by ethical issues and regulatory changes in the EU, the cosmetic industry is focused on finding nonanimal approaches to assess the sensitizing potential of chemicals. Due to the complexity of the sensitization process it is now commonly agreed that hazard identification and risk assessment could only be addressed by combining a battery of assays. Such a combination should face the physicochemical diversity of cosmetic ingredients as well as the need to quantify the skin sensitization potency of chemicals to inform future risk assessment decisions. Among the in vitro methods so far available for pre-validation studies, we developed the Myeloid U937 Skin Sensitization Test (MUSST) based on the induction of CD86 on U937 cells. More recently, we enlarged our set of assays by incorporating the well-described direct peptide reactivity assay (DPRA). In this study we will present how in silico tools, DPRA, MUSST could be jointly used for the evaluation of a particular type of ingredients: we show the results obtained on a benchmark of 25 well known preservatives. This pragmatic exercise, allowed us to get an insight into the feasibility, but also the actual limitations of using nonanimal methods for the screening and/or evaluation of new chemicals for skin sensitization potential. This case study shows that in silico and in vitro tools can already be used, to determine profiles that correspond to « risk categories » based on benchmark chemicals in a defined ingredient class or chemical class family.

PS 2571 IL-18 SECRETION AS A MARKER FOR IDENTIFICATION OF CONTACT SENSITIZERS IN THE EPIDERM *IN VITRO* HUMAN SKIN MODEL.

W. Deng, J. Oldach, A. Armento, S. Ayehunie, H. Kandarova, S. Letasiova, M. Klausner and P. J. Hayden. *MatTek Corporation, Ashland, MA.*

Assessment of the allergic potential of chemicals has traditionally been conducted in animal models such as the local lymph node assay (LLNA). However, recent legislation has prohibited the use of animals for conducting such tests on cosmetics or cosmetic ingredients. Thus, animal alternative tests for contact sensitization are urgently needed. Interluekin-18 (IL-18) secretion has recently been identified as a useful endpoint for determination of contact sensitization potential in keratinocyte monolayer cultures (Naik, SM, et al, J. Invest. Dermatol. 113:766–772, 1999; Corsini, E., et al., Toxicology In Vitro 23: 789-796, 2009). The irritation potential of a chemical may also influence the potency of sensitizing potential. Because epidermal irritation and sensitization are often dependent on chemical penetration and metabolism in the skin, 3D organotypic skin models that possess in vivo-like barrier properties and metabolizing capabilities may provide significant benefits over monolayer culture models for determination of contact sensitization potential. The goal of the current work was to evaluate IL-18 as an endpoint for sensitization potential after topical application of chemicals to the EpiDerm in vitro human skin model. Eleven contact sensitizers and 4 non-sensitizer chemicals were tested based on the chemical set specified by Corsini et al, 2009. A protocol was developed using aqueous or ethanol based vehicles for topical application. Preliminary range-finding experiments were conducted to determine chemical toxicity profiles using the MTT viability assay. Additional doses were then chosen for definitive toxicity and IL-18 secretion experiments. EpiDerm tissue viability and IL-18 secretion by ELISA were evaluated 18-24 hours following topical application of test chemicals. 10/11 contact sensitizers were correctly identified by the assay (90.9% sensitivity) with no false positive results (100% specificity). Thus, the EpiDerm IL-18 assay appears to be a promising tool for in vitro determination of contact sensitization potential.

PS 2572 INCREASED ARSENIC BIOACCESSIBILITY DUE TO MICROBIAL ACTIVITY DOES NOT AFFECT ARSENIC BIOAVAILABILITY.

B. Laird[1], T. Van De Wiele[2], M. Dodd[4], S. Casteel[3] and S. D. Siciliano[1]. *[1]University of Saskatchewan, Saskatoon, Saskatchwean, Canada, [2]Ghent University, Ghent, Belgium, [3]University of Missouri - Columbia, Columbia, MO and [4]Royal Roads University, Victoria, BC, Canada.* Sponsor: L. Weber.

Risk assessors use gastrointestinal (GI) models to estimate the percent of an ingested contaminant solubilized into GI fluids for the calculation of in vitro bioaccessibility (IVBA). In contrast to IVBA, bioavailability (RBA) refers to the percent of an ingested contaminant that is absorbed across the GI epithelium of a mammalian animal model and thereafter reaches systemic circulation. Thus, incorporating IVBA and RBA into exposure assessments enables risk assessors to account for limitations on the release and subsequent absorption of contaminants following the incidental ingestion of soils. However, it is as of yet unknown whether it is necessary to include a microbially-active colon stage for evaluations of contaminant bioaccessibility. The first objective of this research was to assess whether microbial activity in the distal small intestine affects As solubilization from ingested soils. The second objective was to determine whether differences in GI microbial communities result in differences in the intestinal absorption of ingested soil-borne As. Bioaccessible As was extracted from soils under in vitro conditions representative of those found in the distal small intestine with and without microbial activity. Additionally, juvenile swine, which possess similar GI tracts to human children, were treated with soil-borne As with and without oral antibiotics co-administration. Intestinal As absorption with and without antibiotic co-treatment was monitored according to the Urinary Excretion Factor (UEF). In vitro GI microbial activity in the distal small intestine increased As release from contaminated soils. However, these effects were unlikely to be relevant since they were transient and demonstrated small effect sizes. In vivo arsenic absorption for juvenile swine was unaffected by antibiotic treatment. These studies indicated that in vitro GI models need not include microbial activity when calculating As IVBA for human health risk assessment.

PS 2573 AN INTEGRATED APPROACH TO THE RISK ASSESSMENT OF DRUG PRODUCTS.

M. Derzi[1] and R. Naven[2]. *[1]DSRD, Pfizer, Cambridge, MA and [2]CSPG, Pfizer, Groton, CT.* Sponsor: Y. Will.

The ability to identify chemicals with the potential to cause safety issues is important to any risk assessment. For those chemicals with insufficient toxicological data, thresholds of toxicological concern (TTCs) have been developed which define the limits of dietary intake that are considered to be of no concern to human health. The TTC concept has already been applied to the risk assessment of food contaminants and flavoring substances and we have investigated the application of such methodology to the integrated risk assessment of residual solvents, leachables and extractables. Using compounds already classified by the ICH and internal toxicity assessments, we will demonstrate the advantages and limitations of the TTC approach and, in particular, the applicability of the Cramer decision tree which was originally used by Munro and co-workers to determine the thresholds currently used in the TTC. Although the Cramer decision tree requires updating and refining to reflect modern-day chemical space, as part of an integrated risk assessment strategy, it is effective at identifying compounds of low-risk and can be used to establish safe levels greater than those currently employed by the TTC.

PS 2574 PARP-1 ACTIVITY IS INVOLVED IN THE SOLAR UV-INDUCED CUTANEOUS INFLAMMATORY RESPONSE IN THE EPIDERM-FT *IN VITRO* HUMAN SKIN MODEL.

H. Kandarova, A. Armento, J. Oldach, C. Cooney, G. Stolper, M. Klausner and P. J. Hayden. *MatTek Corporation, Ashland, MA.*

Poly (ADP-ribose) polymerase 1 (PARP-1) is known to be activated under conditions of genotoxic stress. In the presence of DNA damage, PARP-1, a nuclear zinc-finger DNA-binding protein, is activated and catalyses the transfer of ADP-ribose from NAD+ to itself (automodification) and a range of other nuclear proteins. UV-induced cutaneous inflammation has been demonstrated in several models and appears to occur through the activation of nuclear factor kappa B (NF-κB). In the present study, The EpiDerm-FT in vitro human skin model (EFT-400) was used to gain insight into the mechanisms of the UV-induced inflammatory response of human epidermal tissue. The EFT-400 model consists of a 3-demensional, highly differentiated epidermis made up of normal human epidermal keratinocytes cultured on a collagen dermis populated with normal human dermal fibroblasts. The model exhibits stratified epidermal components and a fully developed basement membrane at the dermal/epidermal junction. 24 hrs following exposure to 200 or 225 mJ/cm2 simulated solar UV, a dose dependent induction of apoptotic sunburn cells and cyclopyrimidine dimers was observed by histological and immunohistochemical analysis. UV exposure also caused the induction of several inflammatory genes at the mRNA level including a 5.7 fold increase of colony stimulating factor 2 (CSF2), a 2.5 fold increase of interleukin (IL)-1α, a 3.7 fold increase of IL-1α and a 2.3 fold increase of IL-8. PARP-1 inhibition by pre-treatment of the tissue with 20μM 4-amino-1,8-naphthalimide attenuated the increased expression of these genes. UV exposure also caused increases in IL-6, IL-8, G-CSF, GM-CSF, RANTES and IP-10 production at the protein level which were attenuated by PARP-1 inhibition. Looking at NF-κB activation, it was observed that PARP-1 activity was involved in the UV-induced activation of NF-κB as PARP-1 inhibition down-regulated NF-κB activity. Taken together, these results suggest a role for PARP-1 activity in the UV-induced inflammatory response in EpiDerm-FT.

PS 2575 TIMECOURSE OF HEALING AFTER UV-INDUCED DAMAGE IN THE EPIDERM-FT *IN VITRO* HUMAN SKIN MODEL.

G. Stolper, C. Cooney, A. Armento, J. Oldach, S. Letasiova, M. Klausner and P. J. Hayden. *MatTek Corporation, Ashland, MA.*

Solar UV radiation is known to produce deleterious effects on human skin. UVB (280-320 nm) and UVA (320-400 nm) have been shown to cause direct DNA damage, and indirect DNA damage and cytotoxicity via formation of reactive oxygen species, respectively. In the present study, an in vitro human skin model (EpiDerm-FT) was compared to excised human skin to gain insight into the temporal response of human epidermal tissue to UV-induced damage. Normal human keratinocytes were cultured on a normal human fibroblast populated collagen dermis to produce the highly differentiated full-thickness skin model. A solar simulator was used to deliver a range of UVR doses to EpiDerm-FT and excised human skin. Apoptotic sunburn cells were observed in both tissues at 24 hrs post-irradiation. This was followed by progressive epidermal degeneration, inhibition of proliferation and accelerated differentiation of suprabasal cells at days 2-4 post-irradiation. Epidermal proliferation resumed at day 5 and epidermal regeneration continued to day 7. A similar timecourse of epidermal degeneration, proliferation and regeneration was observed in excised human skin. Analysis of EpiDerm-FT culture media demonstrated an initial IL-8 release which decreased to control levels by day 5 post-irradiation. Increased MMP-1 activity was also observed beginning at day 1 post-irradiation and remained elevated throughout the duration of the 7 day experiment. Immunohistochemical analysis revealed cyclopyrimidine dimer (CPD) formation in >90% of basal keratinocyte layers in EpiDerm-FT immediately following UVR exposure. CPD levels decreased with time through day 7 post-irradiation. Ki67 staining demonstrated a dose dependent arrest in basal keratinocyte proliferation at day 1 post-irradiation followed by an elevated rate of proliferation thereafter. Proliferation peaked at day 5 post-irradiation before returning to near control levels at day 7. Our study indicates that EpiDerm-FT is a useful model for study of UV-induced epidermal wound healing as it correlates well to excised human skin.

PS 2576 A FULLERENE DECREASED ORGANOPHOSPHATE-INDUCED TOXICITY IN MICE.

M. Ehrich[1], K. Fuhrman[1], J. Hinckley[1], B. S. Jortner[1], S. Werre[1], R. Van Tassell[2], Z. Zhao[3] and C. R. Kepley[3]. *[1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, [2]Luna Innovations, Blacksburg, VA and [3]Luna NanoWorks, Danville, VA.*

A gadolinium-containing C80 TrimetasphereTM fullerene (Gd3N@C80-OH; GdTMS) was examined for ability to protect mice against clinical signs and acetylcholinesterase (AChE) inhibition that followed ip administration of the organophosphate (OP) diisopropyl phosphorofluoridate (DFP). With DFP (2 or 1.7 mg/kg in 2% ethanol), GdTMS (8 or 24 mg/kg) protected mice from nonlethal doses after 15 min preincubation. Clinical effects of DFP were seen, but atropine treatment was not required. Upon sacrifice 30 min later, improvement in mouse brain AChE activity after 2 mg/kg DFP + GdTMS averaged 10%. Beneficial effect of GdTMS on brain AChE activity was more notable with 1.7 mg/kg DFP, with mean AChE activity >20% higher in brains of 13/18 (72%) fullerene-treated mice ($p < 0.05$). Clinical scores of the DFP-treated mice used for the AChE data (n=8-12) showed definite differences from vehicle-treated controls. Considering DFP-induced effects, and using Chi Square analyses of quantal scores (e.g., activity, gait, involuntary movements, posture), revealed the differences were $p<0.05$ as early as 5 min after dosing. The GdTMS-treated mice showed more improvement when given 2 mg/kg DFP than when given 1.7 mg/kg DFP, probably because clinical signs with this dose of DFP were more dramatic. With this higher dose of DFP, improvements were seen in gait score ($p = 0.025$), gait description ($p=0.027$), involuntary movements ($p=0.032$), and mobility ($p=0.057$) 15 min after dosing. When

the DFP dose was 1.7 mg/kg, improvements were noted with gait (p = 0.042), activity (p = 0.078), and posture (p = 0.059), with involuntary movements and gait score differences between DFP-treated and DFP + fullerene-treated mice at p = 0.12 and p = 0.20, respectively, 5-30 min after ip dosing. With further development, these initial experiments suggest that fullerenes have potential for use against toxicities induced by OP compounds. Supported by NIH 1U01NS063723.

PS 2577 PROLINE RICH POLYPEPTIDES MITIGATE THE EFFECTS OF WHOLE-BODY IRRADIATION IN MICE.

K. D. Thrall[1], M. Franch[2], J. E. Morris[1] and R. E. Weller[1]. [1]*Battelle, Pacific Northwest Division, Richland, WA and* [2]*North Carolina State University, Raleigh, NC.*

The development of medical countermeasures to mitigate or prevent the effects of ionizing radiation is of great importance. Proline rich polypeptides (PRPs) are small chains of amino acids that regulate the production of cytokines and immune functions in the body, and may repair bone marrow cells damaged by high doses of ionizing radiation. A study was conducted to evaluate the efficacy of PRP to restore hematopoietic stem cells and increase the survival of lethally irradiated male Swiss-Webster mice. Proline rich polypeptide was administered to mice as either a single or repeated dose at a high (20 μg) or low (2 μg) level by intraperitoneal injection pre-, post-, or pre- and post- X-ray irradiation at 6.5 and 7.0 Gy. Hematological parameters measured at interim sacrifices (5 and 12 days post irradiation) showed no statistical increased over animals treated with PRP compared to controls, although 30-day recovery was much greater in treated animals. Over 30 days, animals receiving repeated administration of PRP showed a significant increase in survivability (80%) compared to control animals and animals treated with single doses of material (47%). Importantly, time to mortality was increased by 5-7 days in treated groups.

PS 2578 PROTECTIVE ROLE OF DEFEROXAMINE ON BEHAVIORAL EFFECTS IN TRANSGENIC (TG-2576) MICE EXPOSED TO A HIGH ALUMINUM DOSE.

M. Gómez, J. L. Domingo, T. Garcia, D. Ribes and M. Colomina. *Toxicology and Environmental Health, Universitat Rovira i Virgili, Reus, Spain.*

Aluminum (Al), the most abundant metal in the earth crust, is not an essential element. However, it is well established that Al is potentially neurotoxic, being Al exposure linked to some serious neurological disorders including Alzheimer's disease (AD). Biometal deregulations, metal interactions with Abeta, as well as metal-mediated oxidative stress have been implicated in the etiology of AD. The effectiveness of metal chelators has been investigated as a possible therapy in AD treatment. It is well known that deferoxamine (DFO), a hexadentate trihidroxamic acid, acts as an effective Al chelator. However, nowadays there are no data showing whether DFO can improve the behavioral alterations caused by Al exposure in mammals. In recent years, the use of a transgenic mouse model of AD (Tg-2576) has improved the sensitivity of experimental approaches to the potential relationship between Al and AD. The aim of the present study was to assess in 5-months old Tg2576 mice exposed to a chronic oral dose of aluminum lactate (given through the diet, 1 mg Al/g for 6 months) the effects on learning and memory using a Morris Water Maze task. Wild mice were used as controls. General motor activity and anxiety levels were also evaluated using an open-field test. The Water Maze test consisted of 5 acquisition days (4 trials per day) and a probe test at the end of the last acquisition day. The open-field test showed an increased ratio (total distance traveled in the center/total distance traveled) in DFO-treated animals, indicating an anxyolitic effect of DFO, affecting mainly to wild mice. However, no significant Al effects on general motor activity were found. The Water Maze test did not show DFO effects on learning and memory in Al-treated mice. Significant interactions between genotype and DFO indicate the importance of genotype in evaluating neurotoxic effects of metals and chelating agents.

PS 2579 ARE THE NEUROTOXIC EFFECTS OF MANGANESE DUE TO BLOCKAGE OF POST-SYNAPTIC DOPAMINE RECEPTORS.

M. Nelson, T. Adams, D. Beaubrun, M. A. Carroll and E. J. Catapane. *Biology, Medgar Evers College, Brooklyn, NY.*

Manganese (Mn) causes Manganism by disrupting dopaminergic neurotransmission, but the mechanism is unclear. p-Aminosalicylic acid (PAS) is reported an effective treatment but its mechanism is unclear. Lateral cilia of gill of *Crassostrea virginica* are controlled by serotonergic-dopaminergic nerves from their ganglia. Dopamine (DA) produces cilio-inhibition, serotonin (HT) cilio-excitation. Previous work showed Mn blocks cilio-inhibition of DA and is prevented by PAS. We now studied if Mn is blocking DA post-synaptic receptor binding and if PAS prevents Mn from doing this. We observed membrane potentials of lateral ciliated cells using DIBAC, a voltage sensitive fluorescent dye, while measuring cilia beating rates. Applying HT to gill or 5 Hz electrical stimulation (ES) to the branchial nerve caused prolonged membrane depolarization and increased cilia beating rates. Applying DA or 20 Hz ES after exciting cilia repolarized the cell and decreased beating rates. Mn prevented the cilio-inhibition and repolarization. This was prevented when gills were co-treated with PAS. Adding ATP to gill increased cilia beating rates without changing membrane potential. Applying MDL, an adenylcyclase inhibitor, after Mn decreased beating rates without affecting membrane potential. The study shows a correlation between membrane potential of lateral cells and cilia beating rates. It also helps elucidate the neurotoxic mechanism of action of Mn by showing the site of action is after the post-synaptic DA receptors because MDL slowed down cilia in the presence of Mn. This information is helpful to understand causes and potential therapeutic treatments of Manganism. This work was supported by 2R25GM06003 of the Bridge Program of NIGMS, 0516041071 of NYSDOE and 0622197 of the DUE Program of NSF.

PS 2580 THE ABILITY OF PAS, ACETYLSALICYLIC ACID, AND CALCIUM EDTA TO PROTECT AGAINST THE TOXIC EFFECTS OF MANGANESE ON MITOCHONDRIAL RESPIRATION AND MEMBRANE POTENTIAL IN THE GILL OF *CRASSOSTREA VIRGINICA.*

C. Saddler, L. Duncanson, J. Joseph, S. Crawford, E. J. Catapane and M. A. Carroll. *Biology, Medgar Evers College, Brooklyn, NY.*

Manganese (Mn) is an essential metal that at excessive levels in brain causes Manganism, which is similar to Parkinsons disease. The mechanism of action is not completely understood but may be due to mitochondrial damage and resulting dysfunction of the brain's dopaminergic system. Previously, we showed lateral cilia of gill of *Crassostrea virginica* are controlled by serotonergic-dopaminergic innervations from their ganglia and Mn treatments disrupts the cilio-inhibitory dopaminergic system. Here we studied effects of Mn on mitochondrial respiration and membrane potential. We prepared mitochondria from gill and studied respiration and mitochondrial membrane potential using the cationic dye TMRM (Tetramethylrhodamine, methyl ester, perchlorate). We also took time lapse micrographs of gill filaments and mitochondrial smears treated with TMRM. Mn caused dose dependent decreases in O_2 consumption, which was blocked by pretreating with calcium disodium EDTA (caEDTA), p-aminosalicylic acid (PAS) or acetylsalicylic acid (ASA). Each partially reversed the toxic effects of Mn when added to Mn treated mitochondria. Mn decreased mitochondrial membrane potentials and was partially blocked by PAS, but not caEDTA. Time lapse photography revealed the fluorescence of specimens treated with Mn dimmed over a 10 minute period indicating a loss of mitochondrial membrane potential. Pretreatment with PAS or ASA prevented the dimming. The study demonstrates that Mn reduces oxygen consumption and disrupts the mitochondrial membrane potential. PAS and ASA protected against both toxic effects and may be a better therapeutic agents than caEDTA in the treatment of Manganism. This work was supported by 2R25GM06003 of the Bridge Program of NIGMS, 0516041071 of NYSDOE and 0622197 of the DUE Program of NSF.

PS 2581 INTERNATIONAL WORKSHOP ON ALTERNATIVE METHODS TO REDUCE, REFINE, AND REPLACE THE USE OF ANIMALS IN VACCINE POTENCY AND SAFETY TESTING.

J. Kulpa-Eddy[1], R. McFarland[2], R. Isbrucker[3], M. Halder[4], H. Kojima[5], B. Jones[6], N. Johnson[6], D. Allen[6], E. Lipscomb[6], S. Morefield[6], W. Casey[7] and W. Stokes[7]. [1]*USDA, Riverdale, MD,* [2]*U.S. FDA, Rockville, MD,* [3]*Health Canada, Ottawa, ON, Canada,* [4]*ECVAM, Ispra, Italy,* [5]*JaCVAM, Tokyo, Japan,* [6]*ILS, Inc., Research Triangle Park, NC and* [7]*NICEATM, NIEHS, Research Triangle Park, NC.*

Vaccines represent a vital tool in the prevention of infectious diseases. However, regulatory testing to meet vaccine lot release requirements can involve large numbers of animals that may experience unrelieved pain and distress. To advance scientifically sound alternative methods that reduce, refine and replace animal use for human and veterinary vaccine potency and safety testing, NICEATM/ICCVAM organized an international workshop in partnership with ECVAM, JaCVAM and Health Canada. Nearly 200 scientists from 13 countries participated in this SOT co-sponsored workshop. Workshop participants identified knowledge and data gaps that need to be addressed to develop alternative methods. They also identified and prioritized research, development and validation activities needed to address these knowledge and data gaps, including the application of new science and tech-

nology to develop improved methods. They agreed that vaccines that use the largest number of animals and that are associated with the greatest pain and distress should be the highest priority for development and validation of alternative methods. High priorities included implementation and further optimization/development activities for alternative methods for rabies and Clostridial vaccines. Participants also emphasized the need to find ways to avoid or minimize challenge testing with live viruses and bacteria that are hazards to laboratory workers or the environment. Ways to promote the increased use of accepted methods were also discussed. Implementation of the workshop recommendations is expected to advance the use and availability of alternative methods for vaccine potency and safety testing while ensuring continued protection of human and animal health. ILS Staff supported by NIEHS contract N01-ES-35504.

PS 2582 ENERGY BALANCE IN MICE CONSUMING A HIGH FAT DIET AND WHEY PROTEIN.

H. G. Shertzer[1], S. E. Woods[1], M. Krishan[1], M. Genter[1] and K. J. Pearson[2]. *[1]Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH and [2]Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY.*

In mice and humans, a high fat (HF) diet results in excessive weight gain, adiposity and secondary metabolic complications associated with increased risk for fatty liver disease and type 2 diabetes. Protein rich diets may improve various aspects of HF diet-mediated metabolic disorders. The objective of this study was to evaluate the influence of whey protein isolate (WPI) on metabolic changes in mice fed a HF diet, in the context of systemic energy balance. C57BL/6J mice received either a normal diet or a HF diet for 11 weeks. A subgroup of mice consuming HF diet was administered 0.1 g WPI/ml drinking water. WPI diminished the HF diet-induced increases in body weight and percent body fat, although caloric consumption was constant in all groups. These results could be explained by the finding that HF diet decreased basal metabolic rate, respiratory quotient, and hepatic mitochondrial respiration, effects prevented by WPI. Furthermore, WPI restored the diminished intake of protein that occurs with the HF diet, and reduced fat and carbohydrate consumption commensurate with the increase in protein. Health implications for WPI in association with a HF diet were reflected in early biomarkers for fatty liver disease and type 2 diabetes. We found that WPI diminished HF diet-associated fatty liver, by reducing the number of hepatic lipid droplets and deposition of nonpolar lipids by 43% and 76%, respectively. Furthermore, WPI improved glucose tolerance and prevented the increase in insulin resistance in mice fed the HF diet. We conclude that in mice receiving a HF diet, consumption of WPI increases basal metabolic rate and blocks the metabolism of dietary lipid. Since WPI ameliorates the deleterious effects of HF diet-mediated hepatosteatosis and insulin resistance, WPI dietary supplements may intervene in the early stages of developing fatty liver disease and type 2 diabetes. (NIEHS Center for Environmental Genetics P30 ES06096)

PS 2583 A COMPARATIVE STUDY OF NICOTINAMIDE AND TAURINE FOR ANTI-DIABETIC AND ANTIOXIDANT ACTIONS IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS.

K. G. Pandya and C. A. Lau-Cam. *St. John's University, Jamaica, NY.*

Nicotinamide (NIC) and taurine (TAU) are two natural compounds possessing biological properties relevant to diabetes mellitus (DM). While NIC has shown potential for preventing the development of DM, TAU has demonstrated insulin-like effects and antioxidant and hypolipidemic actions. On these premises, the present study was designed to compare NIC and TAU for the ability to protect against typical DM-induced biochemical alterations in an animal model. For this purpose, Sprague Dawley rats (225-250 g) were treated with an oral dose of either NIC or TAU (2.4 mM/kg in citrate buffer pH 4.5), followed 45 min later by an intraperitoneal dose of streptozotocin (STZ) (60 mg/kg in citrate buffer pH 4.5). Treatments with NIC or TAU were continued after 1 day and for another 54 days. Control animals received only citrate buffer pH 4.5 each day. On day 57 of the study, the animals were decapitated to obtain blood samples, which were processed for plasma, and the plasma fractions analyzed for glucose, insulin, cholesterol, triglycerides, malondialdehyde, nitric oxide, reduced glutathione, glutathione disulfide, catalase, glutathione peroxidase and superoxide dismutase. STZ-induced DM resulted in marked increases (ranging from 64-330% over control values) in all parameters but reduced glutathione and antioxidant enzyme activities, which were reduced (by 49-65% of control values). A pretreatment and post treatment with either NIC or TAU led to a significant attenuation of the altering effects of DM, with potency differences between these two compounds not exceeding 10% of each other. The only exceptions were the effects on plasma lipids, for which NIC was inactive, and the plasma NO, for which TAU was more potent than NIC. The present results indicate that while NIC and TAU can provide equipotent protection against the development of DM and its biochemical consequences, NIC is less appropriate because of a lack of hypolipidemic effect and a weaker action on NO depletion.

PS 2584 COMPARISON OF THE EFFECTS OF NICOTINAMIDE AND TAURINE IN THE BRAIN AND SPINAL CORD AGAINST OXIDATIVE STRESS BY STREPTOZOTOCIN-INDUCED DIABETES IN RATS.

S. N. Patel and C. A. Lau-Cam. *St. John's University, Jamaica, NY.*

Diabetes fosters a state of oxidative stress manifested by lipid peroxidation, free radical production and the loss of antioxidant defenses. Nicotinamide (NIC) and taurine (TAU) are two natural compounds which, in this laboratory, have shown the ability to attenuate oxidative stress in the central nervous system of rats when acutely administered before a diabetogen such as streptozotocin (STZ). The present study was undertaken to specifically determine the effect of a long term treatment with either NIC or TAU on diabetes-induced oxidative stress in the brain and spinal cord and the extent of such an effect in various brain areas. In the study, Sprague-Dawley rats (225-250 g) were treated with an oral dose of NIC or TAU (2.4 mM/kg) in citrate buffer pH 4.5, followed 45 min later by an intraperitoneal dose of STZ (60 mg/kg in citrate buffer pH 4.5). A treatment with NIC, TAU or NIC + TAU was continued for an additional 54 days. During this time, control animals received only citrate buffer pH 4.5 each day. On day 57, the brains were removed following decapitation and sectioned into portions representing the brain stem, cerebellum, cortex and spinal cord. An aliquot of each brain area was homogenized with PBS pH 7.4 (1:30, w/v) and the supernatant isolated upon centrifugation was used to assay malondialdehyde (MDA), nitric oxide (NO), reduced (GSH) and oxidized (GSSG) glutathione, and the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD). Relative to control samples, diabetes induced MDA formation, which was significant only in the spinal cord and cerebellum, and increased NO and GSSG while lowering the GSH, GSH/GSSG ratio, and activities of antioxidant enzymes in all brain areas. Generally, the cortex was the most affected and the brain stem the least. Although TAU and NIC attenuated the actions of STZ-induced oxidative stress throughout, at the present doses NIC was usually more potent than TAU, especially against changes in the values of NO, GPX and SOD.

PS 2585 PROTECTIVE EFFICACY OF A COMBINATION OF HUPERZINE A STEREOISOMERS AGAINST SOMAN EXPOSURE IN GUINEA PIGS.

Y. Wang[1], Y. Wei[1], S. Oguntayo[1], N. Jensen[2] and M. P. Nambiar[1, 3]. *[1]Closed Head Injury, Walter Reed Army Institute of Research, Silver Spring, MD, [2]Collaborative Research Facility, U.S. Army Medical Research Institute of Chemical Defense, Edgewood, MD and [3]Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD.*

The mechanism of neuropathology after lethal exposure to nerve agents is complex. Optimal neuroprotection requires drugs with multiple properties to protect against seizures, neuronal degeneration and inflammation as well as induction of neuroregeneration. Huperzine A (Hup), a potential Alzheimer's disease drug, has several beneficial effects including modification of beta-amyloid peptide, reduction of oxidative stress, anti-inflammation, anti-apoptosis and regulation of nerve growth factor. Natural [-]-Hup is a reversible AChE inhibitor that restricts its neuroporotective ability for treatment. [+]-Hup A, a stereoisomer, is a poor AChE inhibitor and can be used as both pre-/post-exposure treatments. We proposed that particular combination of [-]-Hup or [+]-Hup, with different degrees of AChE inhibition can protect strongly by both the mechanisms of reversible AChE inhibition and neuroprotection against nerve agents. Efficacy of [+]-Hup and [+]&[-]-Hup was evaluated in a guinea pig model. Animals were pretreated with [+]-Hup (40mg/kg) or pyridostigmine bromide (PB, 26μg/kg, im) and 30 min later exposed to 1.2 X LD50 soman (GD, 33.6μg/kg, sc), and a combination of atropine (2 mg/kg, im) and 2-PAM (25mg/kg, im) 1 min after GD exposure. EEG and ECG were recorded for 24 h. The survival rate for PB group, [+]-Hup group and [+]&[-]-Hup group was 30%, 80% and 100% (n=10) respectively. Compared to PB, pretreatment with [+]&[-]-Hup and [+]-Hup significantly inhibited epileptic EEG powers (n=8, $p<0.005$), reduced seizures, behavioral symptoms and neuropathology. After exposure to GD, AChE activities of blood was higher in animals treated with [+]&[-]-Hup. [+]-Hup or a combination of [+]&[-]-Hup pretreatment is a novel neuroprotectant that can replace PB to minimize neuropathology and neurobehavioral consequences of nerve agents.

PS 2586 ENHANCED NRF2 ACTIVATION ATTENUATES FASTING-INDUCED FATTY LIVER.

J. Xu[1], J. Moscovitz[1], M. Yamamoto[2] and A. L. Slitt[1]. [1]*Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI and* [2]*Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan.*

Non-Alcoholic fatty liver disease (NAFLD) affects 20% of the U.S. population. Approximately 15 to 20% of patients with fatty liver develop a severe form of hepatic disease known as nonalcoholic steatohepatitis (NASH). The high prevalence of fatty liver and NASH warrants research into the signaling mechanisms responsible for hepatic lipid accumulation. Nuclear factor-erythroid 2-related factor 2 (Nrf2, Nfe2l2), a bZIP family transcription factor, is protective against xenobiotic-related toxicity and chemical-induced carcinogenesis. However little information about Nrf2 function on lipid metabolism is available. Male C57BL/6 and Keap1-KD mice were fed ad libitum or withheld food (fasted) for 24 hours to determine whether enhanced Nrf2 activation in liver could prevent fasting-induced steatosis. In C57BL/6 mice, fasting increased hepatic triglyceride content 240%, and serum triglyceride concentrations 21% compared to fed mice. In contrast, Keap1-KD mice were resistant to fasting, with hepatic triglyceride increased 1.3 fold, and serum triglyceride levels decreased about 6% compared to fed Keap-KD mice. Fasting increased serum free fatty acid levels 110% in C57BL/6, but only 61% Keap1-KD mice. Keap1-KD mice exhibit decreased basal expression of FAS, ACC-1 and SCD-1 in liver, genes important for regulating lipid synthesis, compared to C57BL/6 mice. Fasting decreased FAS, ACC-1 and SCD-1 mRNA expression in liver to a much greater degree in Keap1-KD compared to C57BL/6 mice, 54%, 31% and 81% respectively. These data demonstrated that Keap1-KD mice are resistant to fasting-induced steatosis. Future work will focus on the mechanism by which Nrf2 activity modulates fatty acid synthesis gene expression in liver and promotes resistance to fasting. In conclusion, enhances Nrf2 activation by Keap1 knockdown prevents fasting induced steatosis, pointing to an important function on lipid metabolism (NIH 3R01ES016042-02S2).

PS 2587 PROTECTION OF RETINAL VESSELS FROM HYPEROXIC INJURY BY β-NAPHTHOFLAVONE (BNF) IN NEWBORN RATS.

X. Couroucli, Y. W. Liang, L. Wang, W. Jiang and B. Moorthy. *Pediatrics, Baylor College of Medicine, Houston, TX.*

Supplemental oxygen administration, which is routinely encountered in the treatment of premature infants suffering from respiratory distress, contributes to retinopathy of prematurity (ROP). In this study, we tested the hypothesis that exposure of newborn rats to a combination of β-napthoflavone (BNF) and hyperoxia would alleviate retinopathy and abnormal neovascularization compared to those exposed to hyperoxia alone. Newborn Fisher 344 rats were maintained in room air or exposed to hyperoxia ([gt] than 95% O2) for 7 days. Some animals were treated i.p. with BNF (20 mg/kg) or vehicle (saline), once daily for the first 4 days of hyperoxic exposures, and were sacrificed at selected time points after termination of hyperoxia. Vascular densities of flat mounted retinas were assessed. mRNA expression of VEGF, its receptors (VEGFR-1/sFLT-1 and VEGFR-2), and cytochrome P4501B1 (CYP1B1) was determined by real time RT-PCR. Immediately after 7 days of exposure to hyperoxia, we observed severe vaso-obliteration, compared to the hyperoxia + BNF. Seven to thirty days after termination of hyperoxia, the animals displayed abnormal retinal vessels, whereas those given BNF+ hyperoxia showed significantly lesser extent of neovascularization. At the later time points, VEGF mRNA expression in the hyperoxia group was much higher than that of the BNF+ hyperoxia group. On the other hand, the expression of VEGFR-1 and sFLT-1, but not VEGFR2, was upregulated by BNF + hyperoxia, compared to the hyperoxia only group. Interestingly, retinal CYP1B1 expression was also augmented in the BNF + hyperoxia group. Our study supported the hypothesis that BNF protects retinas from oxygen-induced retinopathy, and that upregulation of VEGFR1 as well as CYP1B1 contributes to the retinoprotective effects of BNF. Further studies are needed to elucidate the mechanisms of the protective action of BNF, which could lead to clinical trials in infants who are at risk of developing ROP.

PS 2588 HYPOXIA-ISCHEMIA TRIGGERS BAX PHOSPHORYLATION, TRANSLOCATION, AND RESULTANT CELL DEATH PHENOTYPE.

S. Krishnan[1], M. Gill[2] and R. Perez-Polo[1]. [1]*Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX and* [2]*Northwestern University, Chicago, IL.* Sponsor: J. Ward.

Hypoxia-ischemia (HI) in the neonatal brain can lead to DNA damage, inflammation, and cell death, all of which ultimately produce neurodevelopmental deficits. Neuronal cell death caused by HI is characterized by an array of cell death phenotypes with necrotic and/or apoptotic characteristics, in which the Bcl-2 family of proteins, including Bax, play a role. Here, we use well-established in vivo and in vitro models of neonatal cerebral HI, and standard molecular techniques to investigate whether HI-dependent phosphorylation of Bax at Ser163, Thr167, and/or Ser184 residues determines Bax multi-organelle localization and correlation with organelle-specific cell death signaling after HI. In rotenone-treated SH-SY5Y cells, a significant increase in p-BaxThr167 was found in mitochondrial fractions when compared to control ½ hour after treatment. In vivo, we also found an increase in p-BaxThr167 in the nucleus at 1 hour after treatment, which correlates with a decrease in mitochondrial p-BaxThr167. This may indicate that phosphorylation of BaxThr167 leads to Bax translocation to the nucleus from mitochondria. We also observed an increase of p-BaxThr167 in cytosolic fractions at 1 hour, indicating that phosphorylation of BaxThr167 may be generally responsible for Bax translocation out of mitochondria. Lastly, no significant changes in p-BaxSer163 were observed up to 6 hours after HI. Interestingly, we observed a decreasing trend in cytosolic p-BaxSer163 gradually from 0 to 6 hours, as well as a significant decrease in p-BaxSer163 in the mitochondrial fraction at 6 hours after HI. These data suggest a role for phosphorylation in the translocation of Bax after HI. Understanding the mechanisms of Bax translocation will aid in the rational design of specified therapeutic strategies which could potentially involve altering Bax subcellular redistribution to decrease the irreversible trauma resulting from a prolonged inflammatory response.

PS 2589 ESTIMATING THE GLOBAL PUBLIC HEALTH IMPLICATIONS OF ELECTRICITY AND COAL CONSUMPTION.

J. M. Gohlke[1, 4], R. Thomas[4], A. Woodward[3], D. Campbell-Lendrum[2], A. Pruss-Ustun[2], S. Hales[2] and C. J. Portier[4]. [1]*Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL,* [2]*Public Health and Environment, World Health Organization, Geneva, Switzerland,* [3]*Population Health, University of Auckland, Auckland, New Zealand and* [4]*Laboratory of Molecular Toxicology, NIEHS, Research Triangle Park, NC.*

The growing health risks associated with greenhouse gas emissions has resulted in a global call for development of new energy policies emphasizing efficiency and low-carbon energy cycles, yet the economic and social development often associated with access to affordable energy are important considerations for improving health in developing nations. Here we assess the relationship between electricity consumption and health outcomes. Using time-series datasets across 41 countries with varying development trajectories between 1965 and 2005, an autoregressive model of life expectancy (LE) and infant mortality (IM) was developed based on electricity consumption, coal consumption, and previous year's LE or IM. The model predicts increased electricity consumption is associated with reduced IM for countries starting with relatively high IM (above 100 per 1000 births), whereas LE was not significantly associated with electricity consumption in any of the models. In addition, the model suggests increasing coal consumption that is not explained by electricity use is associated with increased IM and reduced LE. These results are compared with previously published estimates of disease burdens attributable to energy-related environmental factors including indoor and outdoor air pollution, and water and sanitation. In addition, prediction of health impacts from an integrated air pollution emissions health impact model for coal-fired power plants is compared to the time-series model results. Consistency across analyses of historical time-series and health impact models suggest health benefits may be related to increasing electricity consumption in those countries with very high IM, while negative health impacts of coal consumption are predicted across all countries.

PS 2590 SELENIUM FROM OCEAN FISH PREVENTS METHYLMERCURY TOXICITY.

L. Raymond and N. Ralston. *Energy & Environmental Research Center, University of North Dakota, Grand Forks, ND.*

Supplemental selenium (Se) has been known to counteract mercury (Hg) toxicity since 1967, but the mechanisms of this protective effect have only recently become clear. Methylmercury (MeHg) is a highly specific irreversible inhibitor of Se-dependent enzymes (Se-enzymes). Supplemental dietary Se replenishes Se lost to MeHg binding, thereby maintaining normal Se-enzyme activities. Rationale and scope; Our prior animal studies have shown that the normal range of dietary Se (as sodium selenite) is effective in preventing and reversing MeHg toxicity. Since ocean fish are among the richest sources of dietary Se, we hypothesized that their Se contents would protect against the adverse effects otherwise associated with MeHg exposures from seafood consumption. Experimental procedure; In this study, 120 weanling male Long Evans rats were fed diets containing either low or high MeHg (0.5 or 50 nmol MeHg/g). These diets were augmented with either sodium selenite (~0.1, 1.0, or 10 nmol Se/g) or Se from delipidated protein isolates from bigeye

tuna, swordfish, or mako shark (3.5, 2.3, and 2.1 nmol Se/g respectively). Diets were prepared with torula yeast protein or delipidated fish protein isolates added as 10% of the total diet to replace an equivalent amount of torula yeast protein; (2 Hg levels x 6 Se diets = 12 dietary treatments, 10 rats per group). Contributions of MeHg from the tuna, swordfish, and shark supplemented diets were (1.6, 2.3, and 3.6 nmol MeHg/g respectively). Rats were fed low Se torula yeast based diets for 5 weeks to deplete their tissue Se reserves. Rats were then switched to their assigned dietary treatments for the duration of the study. Results; Rats fed high MeHg, low Se diets showed growth inhibition after 4 weeks, and hind limb crossing after 9 weeks. Rats fed all other dietary treatments grew normally and did not show symptoms of MeHg toxicity. Conclusion; The Se in ocean fish prevented MeHg toxicity in rats fed otherwise toxic levels of MeHg, even though ocean fish contributed additional MeHg levels in the diets.

PS 2591 IS CHELATION THE MECHANISM OF ACTION OF P-AMINOSALICYLIC ACID (PAS) IN THE TREATMENT OF MANGANISM?

V. Petion, J. Rios, J. Mayne, J. Hilaire, D. Nicholson, D. Skeete, K. Ruddock, M. A. Carroll and E. J. Catapane. *Biology, Medgar Evers College, Brooklyn, NY.*

Manganese (Mn) is potentially toxic in high concentrations. Mining, welding and steel manufacturing can expose workers to high levels of Mn leading to Manganism, a Parkinsons-like disorder. The mechanism of toxicity is not fully understood and effective treatments are still being developed. Studies indicate the chelator EDTA is effective in alleviating Manganism. Recently another drug, p-aminosalicylic acid (PAS), is being used for treatment. However, the mechanism of action is unclear and is debated whether effects of PAS are due to anti-inflammation or chelation. We used dialysis tubing with a 100-500 MWCO pore size to mimic the passive elements of Mn movements across the blood-brain barrier. Tubing was set up with Mn plus PAS, EDTA, diaminocyclohexane (DACH) or glucose. Samples were taken outside the bags over time. Mn was measured with an Atomic Absorption Spectrometer. Less Mn was found to pass across bags containing Mn and EDTA as compared to control. The amounts of Mn outside of bags containing Mn with PAS or DACH was higher than controls. The paradoxical results with PAS and DACH are most likely explained because the Mn-PAS and Mn-DACH chelation complex is able to pass through the pore size, while the Mn-EDTA complex can not. Therefore, Mn is diffusing out of the bags by 2 driving forces, that driven by the free Mn concentration gradient and that driven by the Mn-PAS or Mn-DACH chelation complex gradients. We also used a spectrophotometric assay to determine Mn chelating properties of PAS. Mn solutions were exposed to varying concentrations of PAS. Free Mn was converted to permanganate. Levels of permanganate were measured spectrophotometrically. Results indicate PAS is an effective chelator of Mn. Increasing concentrations of PAS reduced levels of free Mn in solution. This information can be of importance with respect to passive transport mechanisms of the blood-brain barrier and can help to clarify the mechanism of action of PAS in alleviating the symptoms of Manganism.

PS 2592 NEUROIMAGING OF MANGANESE TOXICITY: THERAPEUTIC EFFECT OF PARA-AMINO SALICYLIC ACID IN A RAT MODEL.

A. Epur[1, 2], J. Xu[1, 2], W. Zheng[1] and U. Dydak[1, 2]. [1]*School of Health Sciences, Purdue University, West Lafayette, IN and* [2]*Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN.*

Excessive exposure to Manganese (Mn) is known to cause parkinsonism. Earlier studies have shown that para-aminosalicylic acid (PAS) effectively alleviates symptoms in Mn-induced parkinsonism patients and reduces Mn levels in the rat brain. To study the underlying mechanism, changes in the neurochemical profile of the rat brain with or without PAS treatment after Mn exposure were studied using magnetic resonance spectroscopy (MRS) at 9.4T. The rats (220g at start) were divided into three groups: Group P (N=8) received 6 weeks of ip Mn injections (6 mg Mn/kg bodyweight, 5 d/wk), followed by 4 weeks of sc PAS injections at 200 mg/kg, 7 d/wk. Group M (N=8) also received 6 weeks of Mn injections, followed by 4 weeks of saline injections. Group C (controls, N=4) received saline injections throughout the study. Short echo-time MRS data was acquired in all animals at baseline, wk 6 and wk 10 in the thalamus, frontal cortex and hippocampus. Our data showed a significant decrease of the antioxidant glutathione (GSH) with Mn exposure in the thalamus ($p<0.01$), while an increase over time was seen in control animals. PAS treatment restored the GSH levels to normal levels as compared to saline injections (group M). In addition, in Mn exposed brains glutamate as well as the neuronal marker N-acetylaspartate (NAA) did not increase at the same rate as in control animals, and NAA even decreased in frontal cortex at wk 6 ($p=0.01$). This deficit in Glu and NAA was *not* restored by PAS treatment. In the hippocampus the osmoregulator taurine was significantly reduced at the end of Mn exposure compared to controls ($p=0.05$), but was restored to control values irrespective of PAS treatment at wk 10. Overall, our findings indicate that Mn interferes with neuronal development in young Mn-exposed rats in an irreversible way. PAS treatment seems to restore certain metabolic pathways, but not all. Further analysis of GABA and other metabolites is in progress. (Supported by a Showalter Trust Award).

PS 2593 DIABETES-INDUCED CARDIAC INFLAMMATION AND PATHOLOGICAL CHANGES WERE PREVENTED BY WHOLE-BODY LOW-DOSE IRRADIATION.

C. Zhang[2, 1], X. Li[2, 1] and L. Cai[1, 2]. [1]*Department of Pediatrics, University of Louiville, Louisville, KY and* [2]*Chinese-American Research Institute for Diabetic Complications, Wenzhou, Jilin, China.*

It has been recognized for a while that low dose radiation (LDR) induces distinct effects from high doses of ionizing radiation. LDR was found to induce cellular stimulating and adaptive responses, shown by increased cell metabolisms, growth and high resistance to subsequent oxidative stress-induced damage. In the present study, novel preventive effects of repetitive LDR exposure on diabetes-induced cardiac inflammation and damage were investigated. C57BL/6J mice were given multiple low doses of streptozotocin (STZ, 60 mg/kg x 6) to generate type 1 diabetes. A week after the last STZ injection, hyperglycemic mice were diagnosed and treated with and without whole-body LDR exposure (25 mGy X-rays) once every two days for 2, 4, 8, 12 and 16 weeks. Diabetes caused significant increases in cardiac inflammation, shown by time-dependent increases in mRNA and protein expressions of interleukin-18 (IL-18), tumor necrosis factor-alpha (TNF-α), intercellular adhesion molecule-1 (ICAM-1), plasminogen activator inhibitor-1 (PAI-1), and monocyte chemoattractant protein 1 (MCP-1). Repetitive exposure of diabetic mice to LDR significantly reduced diabetes-increased cardiac expressions of IL-18, TNF-α, MCP-1 and PAI-1 at both mRNA and protein levels, except for ICAM-1. Furthermore, cardiac histopathological abnormalities, oxidative damage and fibrosis were significantly evident in diabetic mice, but not in diabetic mice with repetitive LDR exposure. These results suggest that whole-body LDR can significantly reduce diabetes-induced cardiac inflammation and associated pathological changes. Therefore, LDR may potentially become a novel approach to prevention of diabetic cardiovascular complications.

PS 2594 SEROTONIN, OXIDATIVE STRESS, AND DOPAMINE PATHWAYS: THE TARGETS FOR AUTISM TREATMENT.

A. L. Palazzolo[1, 2] and E. Demchuk[1]. [1]*Division of Toxicology & Environmental Medicine, ATSDR/CDC, Atlanta, GA and* [2]*Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY.* Sponsor: B. Fowler.

Autism Spectrum Disorder (ASD) is a developmental disorder that initially manifests in the first three years of life in 1/100 children in the United States. Many of these cases are reported to the Interactive Autism Network (IAN), the largest database of autism patient information in the nation. The information in this database was used to categorize and analyze the use of pharmaceutical treatments for autism. Despite progress in identifying candidate genes associated with ASD, to date no clear etiology or causative marker is found. We identified the three most widely manipulated pathways in the pharmaceutical treatment of ASD: oxidative stress, serotonin, and dopamine. We found a statistically significant association between administration of selective serotonin reuptake inhibitors to Asperger's syndrome patients and reported level of improvement (OR=1.9 [1.4, 2.5]). Also, a weak statistical association between administration of antioxidants to autistic disorder patients and the reported level of improvement (OR=1.47 [1.01, 2.12]) was detected. These findings may help in elucidating the biochemical mechanisms of autism. However, found associations, perhaps, are weaker than the patients would desire. The lack of drugs exhibiting a very high level of improvement illustrates the need for more effective treatments for ASD. It is proposed that characterization of the mechanisms of action of the drugs may suggest genes and other factors associated with increased risk for autism. The results from this study may be used for further in-depth analysis of the genetic and environmental causes of ASD.

PS 2595 UNRAVELING THE EFFECTS OF DEVELOPMENT ON THE OLFACTORY SYSTEM IN A BRDU-INDUCED DEVELOPMENTAL DISORDER MODEL RAT.

M. Kuwagata[1, 2], T. Ogawa[2, 3], T. Nagata[1] and S. Shioda[2]. [1]*Toxicology, Hatano Research Institute, FDSC, Hadano, Japan,* [2]*Anatomy I, Showa University School of Medicine, Shinagawa, Tokyo, Japan and* [3]*Anti-Aging, Medicine Funded Research Labs, Showa University School of Medicine, Shinagawa, Tokyo, Japan.*

The thymidine analog 5-bromo-2-deoxyuridine (BrdU) is a well-known marker for proliferating cells. On the contrary, prenatal exposure to BrdU (50 mg/kg, gestation days; GD, 9-15, ip) is reported to induce developmental neurotoxicity (DNT) in rat offspring, leading to locomotor hyperactivity, impaired learning and memory, and

lower anxiety levels. We have previously reported induction of excessive cell death in various fetal brain areas with different sensitivity to BrdU exposure. However, at this stage, we could not clarify the effects of BrdU on the olfactory bulb (OB). Therefore, to examine the effects of BrdU on OB development, we conducted a histopathological examination of the OB on later brain stages, GD20 fetal brain and postnatal day (PD)11 neonatal brain in rat developmental disorder model. In the BrdU over control group, results revealed a thinner mitral cell layer and fewer distributions of tyrosine hydroxylase (TH) immunoreactive cells in glomerular layer of GD20 fetal brain. In PD11 neonatal group, disruption of the mitral cell layer structure was seen. Neurites of TH-positive cells in the glomerular layer showed abnormal spindle extension, suggesting induction of disturbances in synaptogenesis. Furthermore, fewer distributions of parbalbumin (PV), a Ca-binding protein coexisting with GABA neurons, and presence of underdeveloped neurites in external plexiform layer of TH-immunoreactive cells was also observed in the BrdU group. Using PD11 neonatal brains, we previously reported that BrdU affects GABAergic neurons in the cerebral and limbic cortex (the hippocampus, amygdale and entorhinal area). The present findings reinforce the idea that abnormal GABAergic neurons may contribute to BrdU-induced abnormal behaviors in rat.

PS 2596 PHENCYCLIDINE-INDUCED APOPTOSIS AND CHANGES IN GENE EXPRESSION IN POSTNATAL RAT PUPS.

N. V. Sadovova[1], F. Liu[2], X. Zhang[2], L. Shi[3], L. Guo[3], F. Quan[4], Z. Wen[4], C. Wang[2], T. A. Patterson[5], J. P. Hanig[2], M. G. Paule[2] and W. Slikker[2]. *[1]Toxicologic Pathology Associates, Jefferson, AR, [2]Division of Neurotoxicology, NCTR/FDA, Jefferson, AR, [3]Division of System Toxicology, NCTR/FDA, Jefferson, AR, [4]Z-Tech, ICF International Company, Jefferson, AR and [5]CDER/FDA, Silver Spring, MD.*

Repeated administration of phencyclidine (PCP), an N-methyl-D-aspartate (NMDA) receptor antagonist, during development, may result in neuronal damage that leads to behavioral deficits in adulthood. The present study examined the potential neurotoxic effects of PCP exposure (10 mg/kg) in rats on postnatal days (PNDs) 7, 9 and 11 and the possible underlying mechanism(s) for neurotoxicity. Brain tissue was harvested for RNA extraction and morphological assessments. Elevated neuronal cell death was observed in layers II and III of the frontal cortex in PCP treated group, using Fluoro-Jade C staining. More active caspase-3 positive neurons in PCP treated group further confirmed the neurotoxic effects of PCP, indicating PCP induced rat neuron death is apoptosis in nature. RNA was collected from the frontal cortex for DNA microarray analysis and quantitative RT-PCR. Gene expression profiling was determined using Illumina Rat Ref-12 Expression BeadChips containing 22,226 probes. Based on criteria of a fold change greater than 1.4 and a P-value less than 0.05, 19 genes including NMDAR1 (N-methyl-D-aspartate receptor) and four pro-apoptotic genes were up-regulated, and 25 genes including four anti-apoptotic genes were down-regulated, in the PCP-treated group. Quantitative RT-PCR confirmed the microarray results. These findings support the hypothesis that neurodegeneration caused by PCP occurs, at least in part, through the up-regulation of NMDA receptors, which makes neurons possessing these receptors more vulnerable to endogenous glutamate. Supported by NCTR/NTP IAG # 244-07-0007 (E-2155).

PS 2597 GENE EXPRESSION IN KETAMINE-EXPOSED RAT BRAIN.

C. Wang[1], Q. Shi[2], L. Guo[3], T. A. Patterson[1], S. Dial[2], Q. Li[4], N. Sadovova[5], X. Zhang[1] and J. P. Hanig[6]. *[1]Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR, [2]Division of Systems Toxicology, National Center for Toxicological Research/FDA, Jefferson, AR, [3]Division of Biomedical Toxicology, National Center for Toxicological Research/FDA, Jefferson, AR, [4]Microarray Core Facility, UTSW Medical Center, Dallas, TX, [5]Toxicologic Pathology Associates, Jefferson, AR and [6]Center for Drug Evaluation and Research/FDA, Silver Spring, MD.*

Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, is associated with accelerated neuronal apoptosis in the developing rodent brain. Here, postnatal day (PND) 7 rats were treated with 20 mg/kg ketamine or saline in six successive doses (sc) at 2-h intervals. Brain frontal cortical areas were collected 6 h after the last dose and RNA isolated and hybridized to the Illumina Rat Ref-12 Expression BeadChip with 22,226 probes. Many of the differentially expressed genes were associated with cell death or differentiation and receptor activity. Ingenuity Pathway Analysis software identified perturbations in NMDA-type glutamate, GABA and dopamine receptor signaling. Q-PCR confirmed that NMDA receptor subunits were significantly up-regulated. Up-regulation of NMDA receptor mRNA signaling was further confirmed by in situ hybridization. These observations support our working hypothesis that prolonged ketamine exposure produces up-regulation of NMDA receptors and subsequent over-stimulation of the glutamatergic system by endogenous glutamate, triggering enhanced apoptosis in developing neurons.

PS 2598 MIDAZOLAM-INDUCED CHANGES IN GENE EXPRESSION IN POSTNATAL RAT PUP BRAIN.

F. Liu[1], S. W. Rainosek[2], J. Zhang[3], L. Guo[4], L. Shi[5], N. Sadovova[6], M. G. Paule[1] and W. Slikker[1]. *[1]Division of Neurotoxicology, National Center for Toxicological Research, Jefferson, AR, [2]UAMS College of Medicine, Little Rock, AR, [3]Z-Tech, an ICF International Company at NCTR, Jefferson, AR, [4]Division of Biomedical Toxicology, NCTR/FDA, Jefferson, AR, [5]Division of System Toxicology, NCTR/FDA, Jefferson, AR and [6]Toxicologic Pathology Associates, Jefferson, AR.*

Midazolam is a short-acting drug in the benzodiazapine class, often used for induction and maintenance of anesthesia in combination with either N-methyl-D-aspartate (NMDA) receptor antagonists or other γ-Aminobutyric acid (GABA) receptor agonists in pediatric anesthesia. Little is known regarding its effects on the developing brain. In the present study, postnatal day (PND) 7 rats were treated subcutaneously with 9 mg/kg midazolam or saline at 2-h intervals for 3 times. Brain frontal cortical areas were collected 6 h after the last dose and RNA was isolated for gene expression analysis. Gene expression profiling was determined using a whole genome-wide expression array, Illumina Rat Ref-12 Expression BeadChip with 22,523 probes. The differentially expressed genes (DEGs) were selected using the criteria of a fold change greater than 1.4 and a P-value less than 0.05 comparing the treatment group to the control group. Based on these criteria, 93 genes were up-regulated, and 121 genes were down-regulated in midazolam-treated animals. These DEGs were then searched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) for pathway identification. Twelve pathways were significantly altered by treatment with midazolam. Functionally, these signaling pathways belong to the following 4 categories: 1) metabolism, 2) transcription/genetic information processing, 3) cell death and growth/cellular processes, and 4) neurodegenerative diseases/human diseases. This gene expression analysis provides novel molecular insights into the effects of midazolam in the developing brain.

PS 2599 DIFFERENT APOPTOTIC RESPONSE IN MOUSE BRAIN AFTER NEONATAL EXPOSURE TO DIFFERENT FLAME RETARDANTS.

H. Viberg. *Department Environmental Toxicology, Uppsala University, Uppsala, Sweden.*

Polybrominated diphenyl ethers (PBDEs) and tetrabromo bisphenol A (TBBPA) are used as flame retardants and are detected in environment, wildlife species and human tissues. Earlier studies have shown that exposure to PBDEs during the brain development in the neonatal period affects behavior, learning and memory in adult life, while exposure to TBBPA during the neonatal period did not affect behavioral variables in the adult mouse. PBDE 99, but not TBBPA, has also been shown to affect proteins important for normal brain development neonatally, for example CaMKII, GAP-43 and synaptophysin. There are indications that one of the neurotoxic mechanisms of PBDEs can be apoptosis and therefore we investigated apoptotic markers in neonatal mouse brain. Mouse pups, age 10 days, were exposed to PBDE 99 (12 mg/kg bw, po), TBBPA (11.5 mg/kg bw, po) or ketamine (50 mg/kg bw, sc) and sacrificed 24 or 48 hours after exposure. The brains were sliced and immunohistochemical analysis of caspase-3, caspase-9 and p53 were performed. After slicing the whole brain, a screening analysis were done for caspase-3 and 3 different levels were chosen for semi-quantitative analysis of caspase-3, caspase-9 and p53. So far these analysis have shown that the number of caspase-3 activated cells are significantly increased 24 h after PBDE 99 exposure (100%) and 24 h after ketamine exposure (600%) in two different structures in the neonatal mouse brain. 48 hours after exposure to PBDE 99 or ketamine, there were no significant differences seen. TBBPA did not increase the number of caspase-3 activated cells at any time. These results confirm that PBDE 99 and ketamine can act as neurotoxicants, possibly by inducing apoptosis and altering the levels of proteins important for normal brain development, which may be related to the behavioral and cognitive alterations earlier seen after neonatal exposure to PBDE 99 and ketamine. The lack of effect of TBBPA on caspase-3 activation further strengthen previous results, where TBBPA have been shown not to induce neurotoxic effects on behavior and protein levels after neonatal exposure.

PS 2600 SEX SPECIFIC EFFECTS ON NEUROGLIAL DEVELOPMENT FOLLOWING DEVELOPMENTAL EXPOSURE TO PCBs: IMPLICATIONS FOR ADHD RISK IN MALES.

R. F. Seegal[1, 2], K. Andrews[1], S. Sanchez-Morrissey[1], K. O. Brosch[1] and V. M. Miller[1, 2]. *[1]Wadsworth Center, New York State Department of Health, Albany, NY and [2]School of Public Health, University at Albany, Albany, NY.*

Developmental exposure to environmental toxicants, including polychlorinated biphenyls (PCBs), is associated with an increased risk of ADHD which is four-fold more likely in boys. Hypothyroidism is also a risk factor for ADHD and PCBs sig-

nificantly reduce circulating thyroid hormones. Imaging studies implicate the corpus callosum and the cerebellum in the pathophysiology of ADHD with the later structure contributing to the control of cognitive function. We asked whether gender and PCB exposure interact to alter the developmental trajectory of oligodendrocyte precursor cells (OPCs) in the cerebellum into either oligodendrocytes, by measuring myelin basic protein (MBP), or astrocytes, by measuring glial fibrillary acidic protein (GFAP). We exposed timed pregnant Long Evans dams to PCBs from gestational day 6 until weaning and obtained (i) sera for measurement of circulating thyroxine (T4) and IL-6 levels, the latter implicated in white matter development, and (ii) cerebellar tissue from both male and female offspring at 7, 14, 21 and 42 days of age. Although PCBs significantly reduced T4 levels, they did so equally in male and female offspring. Both IL-6 and cerebellar MBP levels were significantly elevated only in males. While cerebellar GFAP levels were lower in PCB exposed males and higher in females. These data demonstrate that PCBs alter IL-6 and differentiation of OPCs in a sex specific manner leading to greater expression of cerebellar MBP in males and GFAP in females. These findings may aid in understanding the role of environmental contaminants in the etiology of ADHD and elucidate potential mechanisms responsible for the greater incidence of ADHD in boys. Future studies will examine interactions between thyroidal and gonadal hormones and cytokines in the etiology of ADHD and related disorders. Supported in part by NIEHS grant ES015688-01 to RFS.

PS 2601 PRE-PULSE INHIBITION AND THE STARTLE RESPONSE IN GENETICALLY DIFFERENT PCB-TREATED MICE.

C. P. Curran, B. Hays, E. Altenhofen, R. Floyd and A. Mynhier. *Biological Sciences, Northern Kentucky University, Highland Heights, KY.*

Polychlorinated biphenyls (PCBs) are ubiquitous industrial chemicals banned in the 1970s due to their wide-ranging toxicity. Children exposed in utero and lactationally have a higher risk of learning, memory, behavioral and auditory deficits. The goal of our research is to identify genes which affect susceptibility to these pervasive pollutants. The liver enzyme CYP1A2 can sequester toxicants such as dioxins and coplanar PCBs. The aryl hydrocarbon receptor (AHR) binds dioxins and coplanar PCBs, initiating transcription of several genes including CYP1A2. Rodent studies have demonstrated that Cyp1a2(+/+) and poor-affinity Ahr^{d} mothers can protect their offspring from developmental dioxin and PCB exposure compared with *Cyp1a2(-/-)* knockout and high-affinity Ahr^{b1} mice. Our previous research found genetic differences in the Acoustic Startle Response in PCB-treated mice when comparing three mouse lines varying at the *Ahr* and *Cyp1a2(-/-)* loci. Our current work extends those findings by using a newly developed mouse line, $Ahr^{d}Cyp1a2$ *(-/-)*, and the background strain Ahr^{b1} *Cyp1a2(+/+)* as a comparison group. Pregnant dams were treated at gestational day 10.5 (GD10.5) and postnatal day 5 (PND 5) with an environmentally relevant mixture of PCBs or the corn oil vehicle. Offspring were tested at 60 days of age. We measured the baseline startle response to a 110db stimulus and examined pre-pulse inhibition (PPI) using pre-pulse tones of 74 and 76db. There was a trend toward significance for a genotype x treatment interaction (P=0.073) with PCB-treated $Ahr^{d}Cyp1a2$ *(-/-)*mice showing an enhanced baseline startle response. All groups showed normal sensorimotor gating; however, PCB-treated $Ahr^{d}Cyp1a2$ *(-/-)*mice had significantly reduced pre-pulse inhibition at the 74dB level compared with corn oil treated controls. The knockout mice also had significantly reduced pre-pulse inhibition compared with wild type mice under both the 74db (P<0.05) and 76db (P<0.001) pre-pulse conditions.

PS 2602 GENETIC DIFFERENCES IN ADULT STRESS RESPONSE FOLLOWING GESTATIONAL AND LACTATIONAL EXPOSURE TO POLYCHLORINATED BIPHENYLS IN MICE.

E. Altenhofen, B. Hays, C. Kamau-Cheggeh, A. Ashworth, J. Phillips and C. P. Curran. *Biological Sciences, Northern Kentucky University, Highland Heights, KY.*

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that are still present in the human food supply decades after being removed from the market. PCBs are known to affect learning and memory in humans with the most serious effects on the developing brain, which is the focus of our work. Our previous studies revealed significant deficits in learning, memory and behavior in high-affinity $Ahr^{b1}Cyp1a2$*(-/-)* knockout mice exposed to PCBs during gestation and lactation compared with PCB-treated Ahr^{b1} *Cyp1a2(+/+)* and poor affinity $Ahr^{d}Cyp1a2$*(+/+)*mice and controls. Prenatal stress produces similar neurological deficits in rodents and an attenuated stress response in offspring during adulthood. PCBs are known to alter levels of glucocorticoid stress hormones; therefore, we hypothesized that PCBs might exert some neurotoxic effects through changes in glucocorticoid signaling. Our current study examined differences in the stress response of adult mice exposed to either PCBs or the corn oil vehicle during gestation and lactation. To determine if there were genetic differences, we examined three lines of mice varying at the *Ahr* and *Cyp1a2* loci. We used a 5-min. restraint stress to produce peak corticosterone (CORT) levels in blood, then measured the decline in CORT levels 60 min. and 120 min. later. PCB-treated $Ahr^{d}Cyp1a2$ *(-/-)*knockout mice had significantly lower CORT levels after the initial stress (P<0.05). Both lines of *Cyp1a2(-/-)*mice had significantly lower CORT levels at the 120 min. time point (P<0.001). There was also a significant sex difference with females having higher CORT levels compared with males of the same genotype and treatment (P<0.001).

PS 2603 GENETIC DIFFERENCES IN PORSOLT FORCED SWIM BEHAVIOR AND PEAK CORTICOSTERONE LEVELS IN PCB AND CORN OIL-TREATED MICE.

C. Kamau-Cheggeh, R. Floyd, E. Altenhofen, B. Hays and C. P. Curran. *Biological Sciences, Northern Kentucky University, Highland Heights, KY.*

Polychlorinated biphenyls (PCBs) are known to cause wide-ranging adverse health effects including learning and memory deficits, behavioral changes and endocrine disruption. The greatest impacts are seen on the developing brain. Individuals exposed to PCBs show different levels of health effects, so we hypothesized that genetic differences are important in PCB toxicity. Two genes involved in the metabolism of PCBs are the aryl hydrocarbon receptor (AHR) and CYP1A2.We modeled human variation in those two genes using *Cyp1a2(+/+)* wild type mice and *Cyp1a2-/-)* knockouts with either the high-affinity Ahr^{b1} or poor-affinity Ahr^{d} allele. Pregnant mice were treated orally with a PCB mixture similar to what humans are exposed to, while controls were treated with the corn oil vehicle. Mice were treated at gestational day 10.5 (GD10.5) and postnatal day 5 (PND5), which is analogous to the second and third trimester of human brain development. Behavioral testing began on postnatal day 60 (PND 60). One male and one female from each litter were tested in Porsolt forced swim with the latency to float and total time immobile recorded during a 6 min test. Peak plasma corticosterone (CORT) levels were measured 15 min after the initiation of stress. Both $Ahr^{b1}Cyp1a2$*(-/-)* and $Ahr^{d}Cyp1a2$*(-/-)* mice had significantly shorter latencies to float compared with $Ahr^{b1}Cyp1a2$*(+/+)* mice (P<0.001) regardless of treatment. Both groups of *Cyp1a2(-/-)* knockout mice had significantly lower plasma CORT levels compared with *Cyp1a2(+/+)* mice (P<0.001). Females had significantly higher CORT levels than males regardless of treatment or genotype (P<0.001). There were significantly decreased CORT levels in PCB-treated mice and a significantly decreased latency to float in PCB-treated knockout mice (P<0.05). However, sex and genotype appear to be the primary factors underlying the observed differences.

PS 2604 DEVELOPMENTAL EXPOSURE TO PBDE 209 ALTERS ADULT SUSCEPTIBILITY TO PARAOXON AND NICOTINE: GENDER AND NEUROBEHAVIOURAL ANALYSIS.

S. Buratovic, A. Fredriksson, H. Viberg and P. Eriksson. *Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden.*

Newborns, infants and children can be indirectly and directly exposed to PBDEs. This exposure coincides with a period of rapid brain development. Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardants in polymers, especially in electric appliances. A concern is that these compounds are present at a higher level in newborns and toddlers than in the average adult individual, especially the highly brominated PBDEs. We have earlier reported that neonatal exposure to toxicants can lead to an increased susceptibility of the cholinergic system at adult age. The present study was undertaken to investigate whether neonatal exposure of male and female mice to PBDE 209 alters the adult susceptibility to the organophosphorous compound, paraoxon, and to nicotine, respectively.. Neonatal, 3-day-old, NMRI mice were exposed to PBDE 209 (2,2',3,3',4,4',5,5',6,6'-decaBDE at 1.4, 6.0 and 14 μmol/kg bw). At two months of age male mice were exposed to paraoxon (0.25 mg/kg bw, every 2nd day for 7 days) and female mice exposed to nicotine. At the age of 2 months male and female mice were observed for spontaneous behaviour in a novel home environment, before and after adult exposure to paraoxon and nicotine, respectively. Adult male and female mice neonatally exposed to PBDE 209 showed significant impaired spontaneous behaviour. Male mice neonatally exposed to PBDE 209 and to paraoxon as adults developed additional defect spontaneous behaviour and lack of habituation. Female mice neonatally exposed to PBDE 209 showed an increased susceptibility to nicotine, where PBDE 209 exposed mice responded with a decrease in activity to nicotine whereas control mice responded with increased activity. The present study shows that PBDE 209 can induce developmental neurobehavioural defects in both male and female mice. Neonatal exposure to PBDE 209 caused also increased susceptibility in adult mice to paraoxon and nicotine. All these effects were dose response related.

PS 2605 CO-EXPOSURE TO RADIATION AND ENVIRONMENTAL TOXICANTS (PBDE 99 AND MEHG) DURING A DEFINED CRITICAL PHASE OF NEONATAL BRAIN DEVELOPMENT ENHANCES COGNITIVE DEFECTS IN ADULT MICE.

P. Eriksson[1], B. Stenerlöw[2], A. Fredriksson[1] and S. Sundell-Bergman[3]. *[1]Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden, [2]Division of Biomedical Radiation Sciences, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden and [3]Department of Soil and Environment, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Organisms, including man, are continuously exposed to low doses of ionizing radiation (IR) as well as to environmental toxicants. Hence, in the process of developing numerical limits for environmental protection, there is a strong need to consider interactive effects between radiation and other environmental stressors. It is known that exposure to IR during gestation can cause developmental neurotoxic effects in mammals. Animal studies have shown that flame retardants, polybrominated diphenyl ethers (e.g. PBDE 99), and methyl mercury (MeHg) can cause developmental neurotoxic effects. Today there is a lack of knowledge concerning effects and consequences from low-dose exposure to IR during neonatal brain development and its interaction with environmental chemicals. Epidemiological studies have indicated that IR exposure to the brain during infancy might deteriorate cognitive ability in adulthood. Ten-day old neonatal NMRI male mice were exposed to a single oral dose of PBDE 99 (2,2',4,4',5-pentabromodiphenyl ether) (0.8 mg/kg bw) or MeHg (0.40 or 4.0 mg/kg bw). Four hours after this exposure the mice were irradiated with 60Co gamma radiation at doses of 0,2 and 0,5 Gy. The animals were observed for spontaneous behaviour at the ages of 2- and 4-months, and swim maze performance at the age of 5 months. Neither the single dose of PBDE 99 (0.8 mg/kg bw), MeHg (0.4 mg/kg bw) nor the radiation dose of 0.2 Gy affected the spontaneous behaviour, but co-exposure to IR and PBDE 99 or MeHg caused developmental neurobehavioural defects. The study shows that co-exposure to IR and environmental toxicants like PBDE 99 and MeHg, during a critical period of neonatal brain development, significantly enhance developmental neurobehavioural defects and with consequence for adult cognitive functions.

PS 2606 PERINATAL EXPOSURE OF MICE TO DIOXIN INDUCES DEPRESSION-LIKE BEHAVIOR IN A LOW-DOSE SPECIFIC MANNER.

A. Haijima, Y. Zhang, T. Endo, M. Kakeyama and C. Tohyama. *Laboratory of Environmental Health Sciences, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.*

The goal of our study is to characterize and clarify the mechanism of developmental neurotoxicity of dioxins. In our recent studies, deficits in fear memory were observed in mice exposed to dioxins in a dose-dependent manner (e.g., Haijima et al., 2010), and that the impaired learning and enhanced perseverative behavior were induced in a low-dose-specific manner (Endo et al., 2010). Here, we studied the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on depression-like behaviors as well as its dose-response relationship in mice. Pregnant C57BL/6 mice were administered TCDD by gavage at a dose of 0, 0.6 or 3.0 μg/kg b.w. on gestational day 12.5. The TCDD dose is approximately 1/300 or 1/60 of the LD50 value. After the mice reached adulthood, they were subjected to a forced swim test (FST) to examine emotional stress responses. The plasma corticosterone level was measured by ELISA at 0, 30 and 120 min after the FST. In the FST, mice perinatally exposed to 0.6 μg TCDD/kg b.w. showed a significantly shorter latency to become immobile and a significantly longer duration of immobility than the control mice, particularly in the first 2 min of the FST, while mice exposed to 3.0 μg TCDD/kg b.w. showed profiles almost indistinguishable from those of the control mice. In addition, mice exposed to 0.6 but not 3.0 μg TCDD/kg b.w. showed high plasma corticosterone level just after the FST. These results suggest that perinatal exposure of mice to TCDD induces depression-like behavior in mice in a low-dose-specific manner.

PS 2607 ASSESSING LATER-LIFE BEHAVIORAL PHENOTYPES IN RESPONSE TO PRENATAL EXPOSURE TO BENZOPYRENE.

M. M. McCallister[1], M. Maguire[2], A. Ramesh[3] and D. B. Hood[4]. *[1]Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN, [2]Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN, [3]Biochemistry and Cancer Biology, Meharry Medical College, Nashville, TN and [4]Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN.*

Benzo(a)pyrene is an environmental contaminant that is a member of the polycyclic aromatic hydrocarbon (PAH) family. It was reported that environmental levels encountered in the air of New York City can affect child IQ scores adversely. These results are comparable to that reported for low-level lead exposure. The hallmark of lead exposure in studies involving children and animals is behavioral deficits. Since in animal studies the most common way to study adverse effects of lead on behavior is operant conditioning, we have used this same behavioral technique in the present study. Animal model studies have shown that prenatal exposure to PAH's causes behavioral deficits accompanied by a downregulation of glutamate receptor subunits. This reduction is postulated to be correlated with impairments in neuronal activity in later life. In this study, we hypothesize that prenatal exposure to benzo(a)pyrene results in deficits in behavioral learning. Timed-pregnant Long Evans Hooded rats were orally exposed to 0, 150, 300, 600 or 1200μg/kg of B(a)P by oral gavage. The disposition of B(a)P to fetal brain was quantified as a function of exposure (E14-E17) during peak periods of neurogenesis for cerebral cortex and hippocampus. Beginning on P90, offspring pups were habituated and tested in a discrimination reversal-operant behavioral paradigm. The results demonstrate a dose-dependent decrease in the ability to learn the reversal task with associated decreases in correct responses and an enhancement in incorrect responses in the exposure cohort. Taken together, these studies provide mechanistic insights into how in utero polycyclic aromatic hydrocarbon exposure contributes to adverse neurobiological outcomes.

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PS 2608 DEVELOPMENTAL IODIDE DEFICIENCY: REDUCTIONS IN THYROID HORMONES AND IMPAIRED HIPPOCAMPAL TRANSMISSION.

M. Gilbert[1], J. Hedge[1], R. Zoeller[2], K. Kannan[3], K. Crofton[1], L. Valentin-Blasini[4], B. Blount[4] and J. Fisher[5]. *[1]U.S. EPA, Research Triangle Park, NC, [2]University of Massachusetts, Amherst, MA, [3]New York Department of Health, Albany, NY, [4]CDC, Atlanta, GA and [5]U.S. FDA, Little Rock, AR.*

Iodine (I) is essential for thyroid hormone (TH) synthesis, and severe iodine deficiency (ID) during development produces neurological impairments. ID may exacerbate the effects of environmental chemicals on the thyroid axis. This study examined graded levels of ID during pregnancy on the thyroid axis, neurobehavior, and hippocampal electrophysiology in exposed offspring. Female LE rats were placed on diets with varying degrees of ID for 6 wks prior to breeding. I was added to a casein-based diet to produce 5 levels from adequate (>200 ng/gm) to deficient (<25 ng/gm)yielding diets containing 1000, 225, 150, 50 and 25 ng I/gm chow. Pups were sacrificed on postnatal day (PN) 4, 14, 21; blood, thyroid gland, brain collected. Dams were sacrificed on PN21 when pups were weaned to standard Purina chow. Spatial and associative learning were tested in adult offspring. Hippocampal synaptic transmission was assessed in adult males in the 225, 50, 25 ng/gm ID groups. Serum T4 was reduced in pups on PN21 (~20 and 45% in the 2 lowest I diets), TSH was modestly increased, and T3 was not affected. Thyroid weight increased in pups on the lowest I diet, with a similar trend in dams. I content in thyroid glands of dams and pups was reduced in all groups relative to control. Thyroid gland T4 content of PN14 pups was reduced in the 3 lowest I diets, and in the 2 lowest diets in dams. Cortical T4 of PN14 pups was similar across groups and no deficits were detected in Morris water maze or fear conditioning. Synaptic inhibition and long-term potentiation were similar across ID groups. However, ID produced impairments in baseline synaptic function assessed by field potentials recorded from the dentate gyrus in vivo. Excitatory postsynaptic potential and population spike amplitudes were reduced to comparable degrees in the 25 and 50 ng/gm diets. Results indicate that ID reduces TH and produces persistent impairments in brain function. (Does not reflect EPA policy)

PS 2609 IMPAIRED PERFORMANCE ON AN OPERANT LEARNING TASK DURING LIFETIME EXPOSURE TO ACRYLAMIDE IN FISCHER 344 RATS.

M. G. Paule[1], C. S. Roegge[1] and J. D. Garey[2]. *[1]Division of Neurotoxicology, National Center for Toxicological Research U.S. FDA, Jefferson, AR and [2]SRC, Inc., Arlington, VA.*

Acrylamide (ACR) has many industrial uses, is formed in high-temperature cooking processes and is detectable in common foods such as coffee, breads, baby food, potato chips and French fries. Human intake is estimated at 0.44 to 2.3 μg/kg/day. Here, pregnant Fischer 344 rats were treated with ACR (0, 0.1, 0.3, 1.0 or 5.0 mg/kg/day) via gavage from gestational day 6 through parturition. After birth, pups were dosed by gavage at the same dose levels through postnatal day (PND) 22, after which dosing continued via the drinking water. Soon after weaning, one male and one female per litter (8-10/sex/treatment group) began training to perform several operant tasks including an incremental repeated acquisition (IRA) task to assess learning. Diet-restricted rats earned food reinforcers during the IRA task by performing a specific sequence of lever presses that changed each test day. Data were

grouped into 9 observation periods (PNDs 25-85, 102-120, 137-155, 193-267, 284-302, 319-337, 375-449, 466-484, 501-519) and accuracy, percent task completed and response rates were analyzed using separate repeated measures analyses. We previously reported learning impairments in the high ACR dose group (5 mg/kg/day) on the IRA task through the first 52 test sessions. However, across further testing, statistically significant impairments in learning became apparent down to the lowest ACR dose tested (0.1 mg/kg/day). ACR exposed groups completed less of the IRA task than control animals beginning on PND 102 and continuing through the remainder of testing. These results show that daily exposure to ACR from implantation through adulthood impairs performance on a learning task in rats as shown here at dose levels approximately 40 times the estimated daily intake for humans. Performance of the IRA learning task has been identified as the most sensitive endpoint in this study. Supported by Interagency Agreement #224-07-0007 between FDA and NIEHS/NTP (JG and CR participated as ORISE fellows).

PS 2610 BIOFUELS HEALTH RESEARCH AT THE EPA: INITIAL STUDIES WITH INHALED ETHANOL IN RATS.

P. J. Bushnell[1], T. E. Beasley[1], W. K. Boyes[1], H. El-Masri[1], P. A. Evansky[1], J. Ford[1], M. E. Gilbert[1], D. W. Herr[1], W. R. LeFew[1], S. A. Martin[1], K. L. McDaniel[1], E. D. McLanahan[2], V. C. Moser[1], D. K. MacMillan[1] and W. M. Oshiro[1]. *[1]NHEERL, U.S. EPA, Research Triangle Park, NC and [2]NCEA, U.S. EPA, Research Triangle Park, NC .*

The Energy Independence and Security Act of 2007 mandates increased use of alternative fuels in the US automobile fleet. Currently, the primary alternative to petroleum fuels is ethanol, and the public health risk associated with adding ethanol to gasoline at concentrations above 10% is uncertain. Potential neurological and immunological effects from developmental exposure to evaporative emissions from ethanol-gasoline blends are particular concerns. We have therefore begun a research effort involving computational modeling and animal experimentation, with the goal of estimating the toxicity of inhaled vapors from gasoline blended with any concentration of ethanol. Modeling approaches include PBPK models parameterized in pregnant rats for inhaled ethanol (SA Martin abstract) and complex mixtures (WR LeFew abstract). The experimental model system involves exposing pregnant female rats to vapors of ethanol and gasoline-ethanol blends from gestational day 9 through 20, quantifying target-tissue doses by analysis of ethanol concentrations, and determining internal dose-effect relationships in their offspring, using behavioral, neurophysiological, and immunological assessments. Initial studies demonstrated that pregnant female Long-Evans rats (N = 8/group) tolerated a high concentration of inhaled ethanol (20,000 ppm, 6 hr/day for 12 days) without apparent distress and delivered viable litters. No significant differences in maternal weight gain, litter size, or offspring brain weight were observed, nor was fear conditioning affected in the pups at post-natal day (PND) 60. However, compared to controls, exposed pups grew more slowly, and motor activity was increased in females at PND 13 and decreased in males at PND 21. These effects remain to be replicated in a larger study with additional exposure concentrations. Future studies will compare dose-effect relationships across a range of gasoline-ethanol blend ratios. This abstract does not reflect EPA policy.

PS 2611 ACETAMINOPHEN METABOLITES MAY MODULATE HIPPOCAMPAL SENSITIVITY TO SEROTONIN AND SOCIAL INTERACTION BEHAVIOR THROUGH CB1 RECEPTORS.

G. G. Gould[1], L. Nguyen[2], T. F. Burke[2], J. G. Hensler[2], K. Treat[1], L. C. Daws[1] and T. Gu[3]. *[1]Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, [2]Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX and [3]Restorative Dentistry, University of Texas Health Science Center at San Antonio, San Antonio, TX.*

Concordant with replacement of aspirin by acetaminophen (paracetamol) to manage pediatric fever, the rate of autism incidence has increased. A parental survey indicates that acetaminophen use after vaccines in infants may increase the risk for autism in some children. Exposure mediated autism susceptibility could stem from gene polymorphisms in key metabolic enzymes and/or neurotransmitter receptor proteins. Abnormalities in serotonin (5-HT) systems are common among autistic individuals. Acetaminophen's analgesic activity occurs through the action of its metabolites at endocanabinoid CB1 receptors, which are unlikely to bind to 5-HT_{1A} receptors. CB1 and 5-HT receptor modulatory interactions have been reported, but details of their nature in the hippocampus and impact on behavior remains unclear. We have examined this interaction in BTBR and 129Sv mice that exhibit social behavior impairments similar to those seen in autism. Through [^{35}S]GTPgS, [^{3}H] 8-OH-DPAT and [^{3}H] CP 55,940 autoradiography, we found that 129Sv mice have 20% higher 5-HT_{1A} receptor density and BTBR mice have 40-80% increases in 5-HT_{1A} and CB1 receptor function (measured by agonist-induced G-protein coupling) in the hippocampal CA1 (N=4). BTBR mice also exhibited increased social behavior with acute administration of the 5-HT_{1A} partial agonist buspirone (2 mg/kg), acetaminophen (100-500 mg/kg) or the cannabinoid receptor agoninst WIN 55,212 (0.1 mg/kg) in three-chamber sociability tests. These data support modulation of 5-HT systems via CB1 receptors and potential for 5-HT system disruption by acetaminophen overdose.

PS 2612 PERSISTENT CNS EFFECTS OF POSTNATAL EXPOSURE TO CONCENTRATED AMBIENT PARTICLES.

J. L. Allen, J. N. Finkelstein, C. J. Johnston, G. Oberdörster and D. A. Cory-Slechta. *Environmental Medicine & Pediatrics, University of Rochester Medical Center, Rochester, NY.*

Increasing evidence suggests that ultrafine particulate matter (UFP) may adversely influence the brain, particularly early in development, a period of brain vulnerability. If so, air pollutants may represent a largely underappreciated contribution to CNS diseases and disorders. That hypothesis was tested in male and female C57BL6 mice exposed to concentrated ultrafine ambient particles (CAPS) at postnatal days (PN) 4-13, with a subset also re-exposed as adults (AD) at 56-60 days of age (PN + AD). Blood corticosterone levels were decreased in PN males 24 hr after the final CAPS exposure, but increased in both PN and PN+AD females, consistent with hypothalamic-pituitary-adrenal (HPA) axis dysfunction. In males, PN alone persistently suppressed overall response rates during acquisition of prototypical fixed interval (FI) operant schedule-controlled behavior; locomotor activity levels measured 2 wks after final CAPS exposure were comparably reduced in PN and PN+AD groups. In AD females, FI overall response rates were increased, whereas locomotor activity levels were reduced in PN+AD, but not PN alone groups, consistent with enhanced effects. Preliminary assessment of learning using novel object recognition suggests impairment in PN females. Glial fibrillary acidic protein immunoreactivity, a marker of astrogliosis measured in mice sacrificed at 62 days of age, was still increased in midbrain, corpus callosum and hippocampus/dentate gyrus of mice (n=2-3/treatment) after PN only CAPS. Collectively, these findings indicate that CAPS can produce, differentially by gender, persistent behavioral, CNS and HPA dysfunction even following PN only exposure. Given that such exposures are essentially life-long, these results also support the potential for air pollution to serve as a risk factor for neurodevelopmental and/or neurodegenerative disorders. Supported by ES001247 and ES019105.

PS 2613 LIFE SPAN ALCOHOL EXPOSURE MONITORED BY FDG BRAIN PET IMAGING. MORE INFORMATION FROM FEWER ANIMALS.

D. De Groot[1], M. Boogaard[1], R. Nederlof[1], M. Berk[1], E. Uitvlugt[2], L. vd Horst[1], M. Otto[1], A. Wolterbeek[1] and E. De Vries[2]. *[1]TNO Quality of Life, Zeist, Netherlands and [2]Groningen University Medical Center, Groningen, Netherlands.* Sponsor: R. Woutersen.

Objective. To investigate whether in vivo FDG PET imaging can detect adverse effects of alcohol consumption on brain development and compare the results with conventional indicators of behaviour; all this within the scope of animal reduction and refinement (animal 2Rs) in (regulatory) animal experimentation. Strategy. A recently developed protocol for an Extended One Generation Reproduction Toxicity Study [OECD EOGRTS] designed to reduce animal experimentation in reproduction toxicity testing without giving in on safety testing for man was applied. Rats were exposed daily via the drinking water (0, 1.5, 4.0, 6.5, 9.0, 11.5, 14%) from 2 weeks pre-mating to postnatal day (PND) 70 of F1-offspring. F1 offspring of all groups (n=10) was tested for motor, neuromuscular and cognitive functioning. Littermates of ethanol dose groups 0, 4 and 9% (n=3-5) were subjected to [18F]FDG microPET imaging on PND 18, 21, 35, 62 to study brain glucose metabolism. Results/discussion. Behavioral testing demonstrated impairment of neuromuscular functioning in higher dose groups (9, 11.5 and 14%), but no loss of cognition. However, FDG brain uptake showed a dose-dependent (4 and 9%) global decrease over time indicative of mental impairment. In addition, 1) decreased relative uptake was observed in striatum (PND 18) and 2) frontal cortex (PND62), and 3) increased uptake in cerebellum (PN 62), pointing at reduced dopamine function and basal ganglia volume (1), loss of reason and inhibition (2)

and impaired coordination of muscle movements like in drunk people, and reduction of cerebellum volume (3). Conclusions: Life span low dose exposure to alcohol causes dose-dependent abnormalities in brain development which are easily detected by in vivo [18F]FDG microPET imaging. Moreover, far more information is obtained with less than half of the number of animals needed for conventional guideline animal experimentation.

PS 2614 APPLICATION OF LONG-TERM AUTOMATED MONITORING OF HOME-CAGE BEHAVIOUR IN DEVELOPMENTAL NEUROTOXICITY STUDIES.

N. Onishchenko, S. Spulber and S. Ceccatelli. *Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.*

There is evidence that acute as well as chronic exposure to environmental pollutants may result in altered circadian patterns of activity and autonomic regulation even in the absence of additional short-term changes of physiological parameters. The vast majority of the experimental studies investigating the long-term effects of exposure to environmental contaminants are based on acute challenges (e.g. observation of locomotor activity in the open field apparatus), in which the rodent behaviour is driven mainly by the response to novelty. Moreover, the interactions between the human experimenter and the animals, which are associated with the test procedures, have effects that are almost impossible to assess or eliminate. To minimize the bias created by the animal responses associated with human interference, as well as by social isolation, a number of automated systems were developed for the analysis of rodent behaviour in the home-cage environment. By using a system that allows continuous monitoring of locomotor activity in the home-cage (TraffiCage™, NewBehavior, CH), we have characterised the circadian pattern of activity in freely moving mice. The analyses performed on long-term recordings (2-14 days), using custom-made routines allow characterising the changes in the circadian activity occurring in mice exposed prenatally and postnatally to environmental contaminants, such as MeHg and PFOS/PFOA. The results have been verified by established tests addressing specific behavioural traits, such as locomotor activity in the open field and social recognition. The long-term observation of home-cage behaviour in undisturbed social environment allows the detection of subtle alterations in circadian rhythms that are predictive for the long-lasting effects of developmental neurotoxicants.

PS 2615 AUTOMATED VIDEO IMAGE ANALYSIS FOR ACTIVITY MONITORING IN AN EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY TEST WITH LEAD ACETATE.

D. J. Esdaile[1], R. Kubaszky[1], T. Hanley[2], D. Minnema[2] and R. Lewis[3]. [1]*Lab. Research Ltd., Veszprem, Hungary,* [2]*Syngenta Crop Protection LLC, Greensboro, NC and* [3]*Syngenta, Jealotts Hill, Bracknell, United Kingdom.*

The "Extended one-generation reproduction study" in rats as proposed by draft OECD guideline and the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI), requires evaluation of Motor Activity by use of an automated system. The equipment used in this study (SMART Video Tracking System, Harvard Apparatus) comprised a camera-DVD video recording system, with an automated image analysis software package to provide quantitative data on motor activity parameters. Each rat was observed in an individual arena for one hour in a darkened quiet environment without influence of human presence. The system has been shown to provide suitable data on rat activity in a validation study where rats of an equivalent age were treated with Caffeine at 2 or 20 mg/kg, or Chlorpromazine at 5 or 20 mg/kg before evaluation. The increased activity with Caffeine and decreased activity with Chlorpromazine demonstrated the system validity for "detecting both increases and decreases in activity" as required by the draft guideline. In the main study, Lead Acetate was provided in drinking water at 0, 100, 800 and 1700 ppm to F0 adult Wistar rats throughout pre-mating, mating, gestation, and lactation, and to the F1 generation to adulthood. The motor activity of F1 generation rats (10/sex/group) was assessed at PND 56-63. Despite neuropathological examination in these animals showing a treatment-related peripheral neuropathology, there was minimal evidence of motor activity effects under the conditions of this study. This outcome showed that in the case of Lead Acetate, detailed neurohistopathology was a more sensitive indicator of neuropathy in the F1 rats than an automated assessment of motor activity at adulthood. However, this integrated developmental neurotoxicity, immunotoxicity and reprotoxicity protocol was shown to be robust in that the neurotoxic effects of the reference test item were identified.

PS 2616 PERIPHERAL NEUROPATHY IN AN EXTENDED ONE GENERATION REPRODUCTIVE TOXICITY TEST WITH LEAD ACETATE.

J. Wright[1], P. Maslej[2], R. Kubaszky[2], D. J. Esdaile[2], T. Hanley[3], D. Minnema[3] and R. Lewis[1]. [1]*Syngenta, Jealotts Hill, Bracknell, United Kingdom,* [2]*Lab. Research Ltd., Veszprem, Hungary and* [3]*Syngenta Crop Protection LLC, Greensboro, NC.*

This work was performed as part of a study designed to demonstrate the utility of an extended one-generation reproduction study in rats (draft OECD guideline and the Agricultural Chemical Safety Assessment (ACSA) Technical Committee of the ILSI Health and Environmental Sciences Institute (HESI). Lead Acetate (0, 100, 800 and 1700 ppm) was provided in drinking water to adult (F0) Wistar rats throughout pre-mating, mating, gestation, and lactation, and to the F1 generation through to adulthood. Progeny (10/sex/group/interval) were sacrificed at 21 and 70 days of age for examination of Central and Peripheral Nervous System tissues after perfusion-fixation in situ, using 10% neutral buffered formalin. F0 rats were sacrificed at the end of the study and similar tissues preserved. The brain was sectioned at varying levels, so that representative functional areas could be fully evaluated. The levels included, rostral brain (behind olfactory lobes), rostral brain (across the caudate putamen), mid brain to include the dentate gyrus, corpus callosum and thalamic nuclei, caudal brain across the cranial colliculi and cerebellum with cerebellar peduncles and pons. Detailed examination of the brain, cranial nerve V, trigeminal ganglion, Cervical, Thoracic and Lumbar spinal cord, spinal nerves and ganglia revealed no evidence of any significant changes. Evaluation of the peripheral nerves (proximal sciatic, sural and tibial nerves) from both hind limbs of all animals revealed an increased incidence and severity of nerve fibre degeneration in the high-dose parents and 70-day pups, with no evidence of changes in 21-day pups. These findings suggest that chronic exposure to lead may result in peripheral neuropathy similar to that observed in Man (Bleecker et al, 2005), suggesting that the rat may be a relevant model. The neurohistopathology evaluations from this extended one-generation study with the reference item Lead Acetate shows the suitability of the protocol for a detailed neuropathological assesment.

PS 2617 EVALUATION OF THE EFFECT OF DONEPEZIL AND CLONIDINE ON LEARNING MEMORY AND STARTLE RESPONSE IN WISTAR RATS USING ANY-MAZE VIDEO TRACKING SYSTEM AND STARTLE MONITOR SYSTEM.

M. V. Patel, S. M. Paneliya and K. Z. Patani. *Toxicology, Jai Research Foundation, Valvada, Gujarat, India.* Sponsor: P. Desmukh.

This study was conducted to validate Any-Maze Video Tracking System and Startle Monitor System by using Donepezil and Clonidine as positive controls to evaluate the effect on learning memory and startle response in Wistar rats. Wistar rats (30 male and 30 female) were randomly divided into six groups (G1 to G6) and each group comprised of five male and five female rats. The rats from G2 and G3 were treated with Celecoxib (100 mg/kg b. wt) orally for 10 consecutive experimental days for induction of dementia of Alzheimer's type. After induction for 4 experimental days; rats from G2 and G3 were treated intraperitoneally with Donepezil at 0.5 and 1.0 mg/kg, respectively during experimental days 4 to 10. Rats from vehicle control group (G1) were treated with water. Day 7 onwards the rats from G1 to G3 were subjected to water-maze for continuous 3 days for learning and 4th day to assess the retrieval of memory. For the Startle monitor system, rats from G5 and G6 were treated intraperitoneally with Clonidine at 0.4 and 0.8 mg/kg, respectively. Rats from Saline control group (G4) received saline as vehicle. Basic habituation session was performed at experimental day 2 using Startle Monitoring System. Toxicity signs or any significant pathological lesion were not observed in the treated rats. No significant difference was observed in the escape latency time of rats from the treatment groups. It was concluded that the treatment with Donepezil at both doses (0.5 and 1.0 mg/kg) successfully led to retrieval the memory in the dementia/Alzheimers induced rats. There was significant reduction in the maximum response of amplitude (Newtons), maximum time of response (ms) and average of the response amplitude for male and female rates from 0.4 and 0.8 mg/kg dose groups during the five consecutive sessions of Basic Habituation, indicating clonidine treatment leading to suppression of Auditory Startle Response.

PS 2618 *IN VIVO* LONGITUDINAL MAGNETIC RESONANCE SPECTROSCOPY OF THE DEVELOPING RAT BRAIN.

S. Liachenko and J. Ramu. *Neurotoxicology, NCTR/FDA, Jefferson, AR.* Sponsor: M. Paule.

Neurotoxicity is an important outcome to consider when assessing new drug safety and environmental toxicity, especially in the pediatric population. Currently, developmental neurotoxicity is often assessed using traditional ex vivo methods such as

histopathological or biochemical evaluations. However, with the rapid development of in vivo imaging techniques, there is a pressing need for characterization of early brain development using these non-invasive methods. In the current study, male Sprague-Dawley rats were repeatedly scanned using magnetic resonance spectroscopy (MRS) from postnatal day (PND) 14 to 84. MRS was performed using a Bruker BioSpec 7T/30 system with a 72 mm bird-cage transmit and a 4-channel phased array receive coil. Animals were anesthetized using isoflurane and body temperature was maintained at 37.1 ± 0.6°C for the duration of the scans. MRS was acquired from two voxels positioned at the left anterior hippocampus (HC) and anterior cingulate cortex (CC) with the time resolution of ~11 min/voxel. Spectra were quantified using LCModel software and the statistical analyses were performed using ANOVA. The concentrations of creatine (Cre), N-acetylaspartate (NAA), glutamate (Glu), glutamine (Gln), GABA, myo-inositol (mI) and choline (Cho) increased while the concentration of taurine (Tau) decreased with age, reaching plateaus at approximately PNDs 40-50. The changes in Cre and mI were more rapid in the HC, and the changes in Tau, Glu, and Gln were more rapid in CC. There were no regional differences in the concentration of Cho, NAA, and GABA. The data suggest that some brain neurochemistry becomes relatively stabilized by PND 40 in rats. Because mI is predominantly linked to glial and Glu/Gln to neuronal activity, the difference in the dynamics of these metabolites likely reflects the heterogeneity of maturation process throughout the brain. These kinds of data may serve important roles in translational developmental neuroscience and as biomarkers for the emerging science of non-invasive imaging/spectroscopy that is currently being developed. (Supported by NCTR/FDA, #P00731)

PS 2619 EFFECT OF LOW GLUCOSE OR HIGH INSULIN CONDITION ON RAT PRIMARY PERIPHERAL NEURON.

T. Kawataki, T. Yamamoto, H. Takatani, M. Wasaki and E. Kume. *Safety Research Laboratory, Mitsubishi Tanabe Pharmacology Corporation, Saitama, Japan.* Sponsor: J. Sugimoto.

There is some evidence for injury of peripheral neuron in rats administered with insulin, but the mechanism of the effect is not well known. Thus, in the present study, to elucidate mechanism(s) of insulin-induced neuropathy, the long-term culture system of the rat superior cervical ganglion (SCG) neurons was established, and the influence of low glucose on the peripheral neuronal cell was analyzed. SCG neurons were collected from newborn of Sprague-Dawley rat and cultured in α-minimal essential medium (α-MEM) or MEM .with poly-L-lysine-coated dish. The culture condition was developed and optimized by the extension of neurites as an index. Using the optimized culture condition, the influence of a low glucose (0 to 5.5 mM) or high insulin level (5 μg/mL) on peripheral neuron was examined. In α-MEM with poly-L-lysine-coated dish, neurites of SCG were extended well, and also the extension of neurites was observed until 25 days after seeding under the condition of co-culture with Schwann cells. In MEM with poly-L-lysine-coated dish, neurites were also extended, but this condition was not applicable for the co-culture with Schwann cells. Subsequently, the influence of the glucose and insulin on the neurites of SCG was evaluated in the presence (α-MEM) or absence (MEM) of co-culture with Schwann cells. Degeneration with a fragmentation of neurites was observed at a few days after culture in the low glucose medium (0.1 mM) both in the presence and absence of Schwann cells. However, clear change was not observed in the cell body except for slightly shrinking. This phenomenon was agreement with denaturing of the peripheral nerve prior to the cell body degeneration in rat treated with insulin. Moreover, the degeneration of neurites or the cell body was not observed in culture with the high insulin condition. In conclusion, we established the method of long-term culture of rat primary peripheral neuron over 25 days, and using these culture conditions, we demonstrated that low blood glucose level can be the cause of peripheral neuropathy in insulin-treated rats.

PS 2620 SYNAPTOGENESIS IN PRIMARY HIPPOCAMPAL NEURONS IS MODULATED BY ETHANOL-TREATED ASTROCYTES.

P. J. Roque[1], L. G. Costa[1], G. Giordano[1] and M. Guizzetti[2]. [1]*DEOHS, University of Washington, Seattle, WA and* [2]*Department of Psychiatry, University of Illinois at Chicago and Jesse Brown VA Medical Center, Chicago, IL.*

In utero ethanol exposure causes long-term neurobehavioral dysfunction. Although the role of astrocytes in neuronal development is now well recognized, their role in mediating the effects of ethanol has not been fully investigated. Here we study the effect of ethanol-treated astrocytes on hippocampal neuron synaptogenesis. Using an *in vitro* co-culture system, primary hippocampal neurons (13 DIC) were incubated for 24 hours with astrocytes pre-treated with ethanol (75 mM). Neurons were labeled for the pre- and post-synaptic proteins synaptophysin and PSD-95. Three-dimensional analysis of confocal images showed that ethanol pre-treatment of astrocytes significantly increased the number of both pre- and post-synaptic puncta, and caused a greater than 4-fold increase in their overlap, suggesting increased synapse formation. Western blot analysis showed that levels of synaptophysin and PSD-95 in neurons were significantly increased by ethanol. As cholesterol-containing lipoproteins released by astrocytes induce synaptogenesis in retinal ganglion cells, and as ethanol has been shown to increase both cholesterol efflux and lipoprotein release from astrocytes, we investigated the effect of lipoproteins on hippocampal neuron synaptogenesis. Neurons treated with cholesterol-containing high density lipoprotein (20 ug/mL cholesterol) displayed an increase in synaptic structure formation to an extent similar to that seen in neurons co-cultured with ethanol-treated astrocytes. These studies suggest that lipoproteins and/or cholesterol induce synaptogenesis in hippocampal neurons. Future studies will investigate whether ethanol-induced lipoprotein and/or cholesterol release from astrocytes is indeed involved in ethanol-treated astrocyte-induced synaptogenesis. Aberrant synaptogenesis induced by ethanol may be responsible for the cognitive and behavioral dysfunction observed in children prenatally exposed to alcohol (Supported by AA008154 and ES07033).

PS 2621 CHRONIC EXPOSURE REDUCES ACUTE DOMIC ACID TOXICITY *IN VITRO* : ROLE OF GLUTATHIONE.

G. Giordano[1], T. J. Kavanagh[1], E. M. Faustman[1] and L. G. Costa[1, 2]. [1]*Department of Environmental and Occupational Health Sciences, University of washington, Seattle, WA and* [2]*Department of Human Anatomy, Pharmacology and Forensic Science, University of Parma Medical School, Parma, Parma, Italy.*

Domoic acid (DomA) is a potent marine neurotoxin. Low concentrations of DomA (50-100 nM) induce oxidative stress-mediated apoptotic cell death in neurons by activating AMPA/kainate receptors. In the present study we investigated the effects of prolonged (10 days) exposure of cerebellar granule neurons (CGNs) to a very low concentration (5 nM) of DomA on the acute neurotoxicity of this toxin. CGNs from wild-type mice and from mice lacking the glutamate cysteine ligase modifier subunit (Gclm -/-) were utilized for this purpose. CGNs from Gclm -/- mice have very low glutathione (GSH) levels and are very sensitive to DomA toxicity. In CGNs from wild-type mice, prolonged exposure to 5 nM DomA did not cause any overt toxicity, but reduced apoptotic cell death induced by acute exposure to DomA (100 nM for 24 h). This protection was not observed in CGNs from Gclm -/- mice. Prolonged DomA exposure increased GSH levels in CGNs of wild-type, but not of Gclm -/- mice. Levels of GCLC (the catalytic subunit of GCL) were increased in CGNs of both mouse strains, while levels of GCLM and activity of GCL were only increased in CGNs of wild-type mice. These results indicate that a prolonged exposure to very low levels of DomA can up-regulate cellular defense mechanisms that afford partial protection toward a higher acute DomA exposure. CGNs from Gclm -/- mice, which are already more sensitive to DomA toxicity, are unable to up-regulate their GSH levels. As Gclm -/- mice may represent a model for a common human polymorphism of GCLM, such individuals may be at particular risk for neurotoxicity of DomA (Supp. in part by NSF).

PS 2622 METHYLMERCURY AND NON-DIOXIN LIKE POLYCHLORINATED BIPHENYLS INTERFERE WITH NEURONAL DIFFERENTIATION OF CORTICAL NEURAL STEM CELLS.

W. Ibrahim[1], R. Tofighi[1], P. Rebellato[2], R. Bose[1], P. Andersson[3], P. Uhlen[2] and S. Ceccatelli[1]. [1]*Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden,* [2]*Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden and* [3]*Department of Chemistry, Umeå Universitet, Umeå, Sweden.*

Methylmercury (MeHg) and polychlorinated biphenyls (PCBs) are food contaminants that have been shown to affect the developing nervous system. They can cross the placenta and are found in breast milk posing a threat to fetuses and infants. In the present study we investigated the effects of MeHg and two non-dioxin-like (NDL)-PCBs (153 and 180) on the spontaneous differentiation process of primary embryonic cortical neural stem cells (NSCs) from rats. After withdrawal of basic fibroblast growth factor (bFGF) from the culture medium to promote NSC spontaneous differentiation, cells were exposed to nanomolar concentrations of MeHg, PCB 153 or 180 for 48 h and allowed to differentiate for five additional days. Exposure to MeHg decreased significantly the number of neurite-bearing Tuj1 positive cells, whereas exposure to PCB 153 or 180 increased it. Further experiments revealed that exposure to MeHg in absence of bFGF stimulates proliferation, as

shown by the increased number of cells positive for EdU-staining and the upregulation of Cyclin D3 protein. Conversely, exposure to PCB 153 or 180 resulted in a lower number of EdU-positive cells with no changes in the expression of Cyclin D3 as compared to control cells. Experiments with Fucci transfection confirmed the observed effects on proliferation. In agreement, MeHg increased the number of NSCs exhibiting spontaneous Ca2+ activity, whereas both PCBs caused a significant decrease. q-PCR analyses pointed to an involvement of Notch signaling in the observed effects of MeHg and PCBs on NSCs differentiation. Alterations in neuronal differentiation may partially explain the adverse impact of MeHg and PCBs on the developing nervous system.

PS 2623 NON-DIOXIN-LIKE POLYCHLORINATED BIPHENYLS (PCBS) ENHANCE DENDRITIC GROWTH IN CULTURED HIPPOCAMPAL NEURONS *VIA* RYANODINE RECEPTOR (RYR)-DEPENDENT ACTIVATION OF CALCIUM-DEPENDENT SIGNALING PATHWAYS.

D. D. Bose[1], D. Yang[1], G. Wayman[2], A. Lesiak[2], D. Bruun[1], I. N. Pessah[1] and P. J. Lein[1]. *[1]Veterinary Molecular Biosciences, University of California, Davis, Davis, CA and [2]Veterinary and Comparative Anatomy, Washington State University, Pullman, WA.*

Exposure of the developing brain to PCBs is linked to cognitive deficits in humans and experimental animals, but the mechanism(s) underlying the developmental neurotoxicity of these compounds remain speculative. Our previous in vivo studies of rats exposed developmentally to PCBs demonstrated that PCBs disrupt spatial learning and memory coincident with altered dendritic growth and ryanodine receptor (RyR) expression. Here we show that PCB effects on dendritic growth and RyRs are causally related. We quantified dendritic growth in cultured hippocampal neurons transfected with a GFP-tagged MAP2 construct following a 48h exposure to either PCB95, a non-coplanar PCB congener that is a potent activator of the RyR, or PCB66 a coplanar PCB congener with no activity at the RyR. PCB95, but not PCB66, significantly enhanced dendritic growth. The dendrite promoting activity of PCB95 was evident at concentrations as low as 2pM and was completely blocked by FLA365, a RyR channel blocker and by siRNA knockdown of RyRs. Acute exposure to PCB95 (200nM) increased the frequency and amplitude of spontaneous Ca^{2+} oscillations in hippocampal neurons whereas no such effect was observed with PCB66 (200nM) and vehicle (DMSO). In neurons exposed to PCB95 for 48hr, the amplitude of evoked Ca^{2+} transients decreased in a concentration-dependent manner. Genetic and pharmacological blockade of Ca^{2+} signaling molecules implicated in activity-dependent dendritic growth inhibited PCB95-induced dendritic growth. These data demonstrate that PCB activation of RyR modulates Ca^{2+}-dependent signaling pathways that regulate dendritic plasticity, and suggest that non-coplanar PCBs may be useful probes for studying the role of the RyR in activity-dependent dendritic growth. This work supported by NIH grants ES014901 (PJL and INP) and MH86032 (GAW).

PS 2624 ENANTIOSELECTIVE EFFECTS OF PCB136 ON DENDRITIC GROWTH ARE RYANODINE RECEPTOR-DEPENDENT.

D. Yang[1], I. Kania-Korwel[2], D. Bose[1], A. Ghogha[1], I. Pessah[1], H. Lehmler[2] and P. Lein[1]. *[1]Veterinary Medicine, Molecular Biosciences, University of California Davis, Davis, CA and [2]Occupational & Environmental Health, University of Iowa, Iowa City, IA.*

Polychlorinated biphenyl (PCB) congeners with multiple ortho chlorine substitutions sensitize ryanodine receptors (RyR), and experimental evidence indicates this activity contributes to PCB developmental neurotoxicity. Many of these ortho-substituted congeners display axial chirality and we have previously reported that chiral PCB atropisomers differentially sensitize RyR. In this study, we test the hypothesis that chiral PCB136 enantioselectively influences RyR-mediated dendritic growth. Primary cultured rat hippocampal neurons were exposed to purified PCB136 atropisomers for 48 hr beginning on day 9 in vitro. (-)-PCB136, which sensitizes RyR, enhances dendritic growth at concentrations ranging from 0.1nM to 1μM, and this effect is blocked by the RyR specific antagonist FLA365. In contrast, (+)-PCB136, which lacks activity towards RyR, has no effect on dendritic growth. Neither atropisomer alters axonal growth or cell viability. Quantification of PCB136 atropisomers in cell media and cell pellets indicates that the enantioselective effects on dendritic growth are not due to enantioselective partitioning of PCB136 into cells. Imaging of cultured hippocampal neurons loaded with the Ca2+-sensitive dye Fluo-4 indicates that (-)- but not (+)-PCB136 increases the frequency of spontaneous Ca2+ oscillations in cultured hippocampal neurons, which is consistent with the finding that (-)- but not (+)-PCB136 increases electrical activity in neurons plated on microelectrode arrays. These data support the hypothesis that enantioselective effects on RyR mediate the enantioselective effects of PCB136 on dendritic growth, and suggest that the significant variability in enantiomeric enrichment of chiral PCBs in the human population may be a significant factor in determining the risk for adverse neurodevelopmental outcomes following PCB exposure (supported by NIH grant ES017425).

PS 2625 POLYCHLORINATED BIPHENYLS (PCBS) ALTER GLUTAMATE RELEASE FROM PRESYNAPTIC NERVE TERMINALS ISOLATED FROM THE RAT HIPPOCAMPUS INDEPENDENT OF ARYL HYDROCARBON RECEPTOR (AHR) MEDIATED GENE TRANSCRIPTION.

N. Newman and F. Schanne. *Pharmaceutical Sciences, St. John's University, Queens, NY.*

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental contaminants known to elicit a myriad of toxicological responses. There are 209 possible PCB congeners, depending upon the number of chlorines present and their position on either of the phenyl rings. PCBs were produced as complex mixtures of dioxin-like coplanar and non-dioxin-like non-coplanar congeners. The coplanar congeners are commonly associated with cancer and other health risks whereas the non-coplanar congeners, especially those that are di-ortho- substituted, are more often associated with neurotoxicity. 3,3′,4,4′-Tetrachlorobiphenyl (PCB 77), a dioxin like meta- and para-substituted co-planar congener, is known to be neuroactive in vitro. It has been suggested that, contrary to the notion that co-planar PCBs require aryl hydrocarbon receptor (Ahr) mediated gene transcription to exert their toxicity, PCB 77 exhibits Ahr independent neurotoxicity. This study analyzed the effects of PCB 77 on the evoked release of glutamate from pre-synaptic nerve terminals (synaptosomes) isolated from the CA1 region of the rat hippocampus. Evoked release of glutamate is central to synaptic transmission and is modified as a component of synaptic plasticity. Release of endogenous glutamate was estimated by measuring the fluorescence of NADPH resulting from the reduction of NADP+ by glutamate dehydrogenase. PCB 77 significantly enhanced glutamate release at micromolar treatment concentrations. Treatment with the non-coplanar di-ortho substituted 2,2',5,5' Tetrachlorobiphenyl (PCB 52) also significantly enhanced glutamate release. However, when these PCBs were combined, glutamate release was significantly diminished suggesting that co-planar and non-coplanar PCBs impact this process via different mechanisms. As synaptosomes are devoid of a nucleus, these observations confirm that the effects of these PCBs on synaptosomal glutamate release are independent of Ahr mediated gene transcription.

PS 2626 NEUROBEHAVIORAL AND TRANSCRIPTIONAL CONSEQUENCES OF ESTROGEN RELATED RECEPTOR GAMMA ACTIVATION BY LOW-DOSE BISPHENOL A EXPOSURE DURING NEUROGENESIS.

K. S. Saili, M. M. Corvi, J. Przybyla, J. K. LaDu, K. A. Anderson and R. L. Tanguay. *Department of Environmental & Molecular Toxicology, Environmental Health Sciences Center, Oregon State University, Corvallis, OR.*

Bisphenol A (BPA) is detectable in the serum and breast milk of pregnant and nursing women. Risk to fetuses and infants is a concern based on evidence from rodent studies that exposure to low levels of BPA during critical periods of central nervous system (CNS) development leads to persistent behavioral impairments. The mechanism by which BPA impairs CNS development is unclear, but likely involves both classical estrogen receptor (ER) and estrogen related receptor (ERR) signaling pathways. BPA's high binding affinity for estrogen related receptor gamma (ERRγ) suggests a central role for this orphan nuclear receptor. To assess the effects of BPA on CNS development, zebrafish were exposed to concentrations below the no observed adverse effect concentration (NOAEC) during early neurogenesis (10–58 hours post fertilization, hpf) and behavior endpoints were assessed at 120 hpf. Increased locomotor activity was measured in larvae exposed to 0.1 μM (corresponding to a human-relevant tissue dose <5 μg/kg). Additionally, larvae exposed to 0.1 μM 17β-estradiol (E2) or GSK4716, an ERRγ agonist, exhibited a hyperactive phenotype. To confirm a role for ERRγ, an antisense oligonucleotide morpholino (MO) was used to knock down endogenous ERRγ expression during BPA, E2, or GSK4716 exposure. While BPA-exposed embryos injected with control MO

exhibited a hyperactive phenotype, behavior in BPA-exposed embryos injected with ERRγ MO was indistinguishable from unexposed control morphants. To further characterize the transcriptional events associated with the hyperactive phenotype, transcripts associated with ER or ERR signaling were measured by qRT-PCR following BPA, E2, or GSK4716 exposure. Taken together, we have identified a role for ERRγ in mediating the neurobehavioral toxicity of developmental BPA exposure. This research was supported in part by NIEHS T32ES7060, ES00210, and an EPA STAR Graduate Fellowship to KSS.

PS 2627 EFFECTS OF RADIO FREQUENCY RADIATION EXPOSURE FROM MOBILE PHONES ON BRAIN MICROVESSEL ENDOTHELIAL CELLS, A MODEL OF BLOOD BRAIN BARRIER: AN *IN VITRO* STUDY.

E. Cuevas[1], S. M. Lantz[1], W. J. Trickler[1], B. R. Robinson[1], G. D. Newport[1], P. C. Howard[2], N. J. Walker[3], M. Wyde[3], A. Desta[4], M. G. Paule[1] and S. F. Ali[1]. *[1]Neurochemistry Laboratory, Division of Neurotoxicology, NCTR/U.S. FDA, Jefferson, AR, [2]Office of Scientific Coordination, NCTR/U.S. FDA, Jefferson, AR, [3]National Toxicology Program, NIEHS, Research Triangle Park, NC and [4]ODE, CDRH/U.S. FDA, Sliver Spring, MD.*

The association between exposure to radio frequency (RF) energy similar to that emitted by mobile phones and potential adverse effects to the central nervous system, including blood-brain barrier (BBB) dysfunction, neurotoxicity and brain tumors continue to be an area of scientific controversy. We have designed studies to test the effects of RF energy exposures on rat and bovine primary brain microvessel endothelial cells (BMEC). BMEC cells were isolated from fresh rat and bovine cerebral cortices and cultured in 35 mm petri dishes (12-14 days). The BMEC (80% confluent) were exposed to RF energy at the specific absorption rate (SAR) levels of 5.04 W/kg (rat) or 5.98 W/kg (bovine) (600sec - 60sec; on-off cycle) for 4, 8, or 24 hours. (The maximum permissible SAR from mobile phones in US is 1.6 W/kg). Cell viability was evaluated by lactate dehydrogenase (LDH) assay and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Nitric oxide (NO) changes were determined by Griess reaction. Heat shock proteins (HSPs), receptor for advanced glycation end products (RAGE) expression, and inflammatory makers [tumor necrosis factor-alpha (TNFα), interleukin 1 beta (IL-1β), interleukin 2 (IL-2), and prostaglandin E2 (PGE-2)] levels were evaluated. Under these conditions, SAR exposures produced no cytotoxic changes as measured by LDH, MTT and NO, however, the same SAR exposure increased activation of HSP27, HSP90, and RAGE, and changes in several inflammatory markers (TNFα, IL-1β, IL-2, and PGE-2) at 8 and 24 hours. These preliminary results indicate that SAR exposure activates HSPs family, neuroinflammatory markers which may reflect early intracellular signaling pathways and may ultimately alter BBB integrity. These studies were supported by NTP/NCTR IAG 224-07-007.

PS 2628 REARING CONDITIONS DIFFERENTIALLY AFFECT THE LOCOMOTOR BEHAVIOR OF LARVAL ZEBRAFISH, BUT NOT THEIR RESPONSE TO VALPROATE-INDUCED DEVELOPMENTAL NEUROTOXICITY.

D. Zellner[1], B. Padnos[2], D. L. Hunter[2], R. C. MacPhail[3] and S. Padilla[2]. *[1]Meredith College, Raleigh, NC, [2]ISTD, U.S. EPA, Res. Tri. Pk., NC and [3]TAD, U.S. EPA, Res. Tri. Pk., NC.*

Zebrafish (*Danio rerio*) are widely used in developmental research, but little is known about the role environment may play in their development. Zebrafish are a highly social organism; thus exposure to or isolation from social environments may have profound effects. Details of rearing conditions are often sparse in the literature. This study compared (1) the activity of larval zebrafish that were raised individually or in groups, and (2) the effect of the developmental neurotoxicant valproate. We randomly assigned embryos to isolative or inclusive social environments from 0 to 5 days post fertilization (dpf), while treating them with or without valproate (50 uM) from 0-2 dpf resulting in a total of four groups (group control, group treated, single control, single treated). At 5 dpf all embryos were transferred to singly-housed environments where they remained through locomotor testing (alternating periods of light and dark) conducted on day 6. Larvae that had been raised in groups had higher levels of activity than larvae that had been raised individually, but only in the dark periods. Valproate increased activity in the dark in both the singly and group housed groups. Further analyses indicated rearing condition did not significantly affect larval responses to valproate. These results indicate that rearing conditions affect behavioral development of zebrafish larvae, but that rearing conditions may not affect the effect of a developmental neurotoxicant. *This is an abstract of a proposed presentation: the information does not necessarily reflect EPA policy.*

PS 2629 ASSESSMENT OF CHEMICAL EFFECTS ON NEURITE OUTGROWTH, NEURONAL POLARIZATION, AND SYNAPTOGENESIS IN RAT CORTICAL NEURONS USING HIGH CONTENT IMAGE ANALYSIS.

B. L. Robinette, J. A. Harrill and W. R. Mundy. *Integrated Systems Toxicology Division, NHEERL, U.S. EPA, Research Triangle Park, NC.*

There is a need for efficient, cost-effective methods for screening and prioritization of potential developmental neurotoxicants. One approach uses *in vitro* cell culture models that can recapitulate the critical processes of nervous system development. *In vitro*, primary cultures of neocortex undergo a series of morphological changes including neurite outgrowth, polariation of neurites and synaptogenesis similar to that observed *in vivo*. In the present work, primary cultures were prepared from rat neocortex in a 96-well plate format. A combination of immunocytochemical labeling and high-content image analysis (HCA) protocols were developed in order to quantify the time course of neurite outgrowth (βIII-tubulin), neuronal polarization (βIII-tubulin and MAP2 or neurofilament) and synapse formation (MAP2 and synapsin). Neurite outgrowth occurred rapidly: 75 % of neurons developed neurites by 24 h. At this time, ~20% of neurons were axon bearing. An increase in total neurite length continued up 5 days *in vitro* (DIV), at which time a change in the cytoplasmic distribution if MAP2 was observed. The ratio of βIII-tubulin and MAP2 labeled neurite lengths (NPR) increased from 1.35 to 2.12 between 24 and 120 h, indicative of neuronal polarization. An increase in synapse number was observed between 6 and 15 DIV. The concentration-response of model chemicals known to inhibit each process was then examined. K252a (0.01 - 1 μM) applied at 2 h inhibited total neurite outgrowth (20 %), specifically axon outgrowth (97 %), at 24 h and inhibited neuronal polarization at 120 h (NPR = 1.3). In addition, mevastatin applied at 9 DIV (0.3 – 30 μM) decreased synapse number at 15 DIV (~35 %). Effects occurred in the absence of overt cytotoxicity. These data demonstrate that HCA using a single culture system can be used to screen for chemical effects on multiple processes of nervous system development. *This abstract does not necessarily reflect USEPA policy.*

PS 2630 INITIATING EFFECTS OF METHYLEUGENOL IN RAT LIVER.

M. J. Iatropoulos, A. M. Jeffrey, J. Duan and G. M. Williams. *Department of Pathology, New York Medical College, Valhalla, NY.*

Methyleugenol (ME), a constituent of basil, is hepatocarcinogenic in rodents. To study initiating effects of ME in rat liver, groups of male F344 rats were administered by gavage 0 (control, C), 62 (low dose, LD), 125 (mid dose, MD), or 250 (high dose, HD) mg/kg bw/dose, 3 times/week for 8 or 16 weeks (providing cumulative doses (CD) of 185, 275 or 750 mg/kg bw/week similar to the weekly CD of the NTP ME bioassay). After week 16, half of the rats were fed 500 ppm phenobarbital in the diet (+PB), and the rest were maintained on C diet (-PB) for 24 weeks. At 8 weeks, the MD and HD groups had bw reductions, and group relative liver weight increases. ^{32}P-nucleotide postlabeling (NPL) revealed 3 adducts in the livers of all ME groups. The hepatic DNA-adduct values were dose related and were increased in all ME groups compared to C. PCNA immunohistochemistry (IHC) revealed that the group hepatocellular replicating fraction (RF) in all ME groups was increased compared to C. Only the HD rats had hepatocellular altered foci (HAF), identified by glutathione *S*-transferase-placental form IHC. At 16 weeks, there were no differences in bw between groups and no changes in measured serum liver enzymes. All ME groups had dose-related increases in DNA adducts in both liver and white blood cells (WBCs) compared to C, although the WBC levels were 1% of those of the liver. The group RF and HAF in all ME groups were increased compared to C in a dose-related pattern. At the end of the promotion/maintenance phase (weeks 16-40), the NPL values in both ME/+PB and ME/-PB groups were lower than the respective 16-wk NPL values, indicating DNA repair. HAF were evident in all +PB groups, including C. The multiplicity and severity of HAF was similar between C (1 and 1+, respectively) and LD (2 and 1+) while those of the MD (8.3 and 3+) and HD (28.8 and 4+) were increased. Hepatocellular adenomas were evident in a dose-related pattern in both +PB and -PB MD and HD groups, attaining higher multiplicity and severity in the +PB groups. Thus, ME had initiating activity and the LD was a NOAEL for pre- and neoplasia, but not for DNA adducts.

PS 2631 LIVER TOXICITY FOLLOWING KAVA KAVA ADMINISTRATION IN F344 RATS AND B6C3F1 MICE.

M. Behl[1], R. S. Chhabra[1], A. Nyska[2], L. Fomby[3], B. R. Sparrow[3], M. R. Hejmancik[3] and P. C. Chan[1]. *[1]NTP, NIH, Research Triangle Park, NC, [2]Timrat and Sackler School of Medicine, Tel Aviv University, Timrat, Israel and [3]Battelle, Columbus, OH.*

Kava Kava is an herbal supplement extensively being used as an alternative to anti-anxiety drugs such as Xanax® and Valium®. It has also been proposed for use to help children with hyperactivity. The reports on hepatotoxicity associated with kava-containing dietary supplements in humans are of widespread concern, resulting in its ban in several European countries. Although the US FDA has issued warnings about the potential hepatotoxic effects of Kava, it continues to be widely used in the United States due to lack of sufficient toxicity data. The purpose of the NTP studies was to characterize the toxicity and carcinogenicity of Kava in male and female F344 rats and B6C3F1 mice. The animals were administered kava root extract orally by gavage in corn oil for two weeks, thirteen weeks or two years.
Results from the 2-week and 13-week studies in rats and mice administered kava at doses of 0, 0.125, 0.25, 0.5, 1.0, and 2.0 g/kg body weight revealed dose-related increases in the incidences of heptocellular hypertrophy. This was accompanied by significant induction of hepatic drug metabolizing enzymes. In the chronic studies, rats were gavaged 0.1, 0.3, 1.0 g/kg Kava and mice with 0.25, 0.5, 1.0 g/kg. Dose-related increases in hepatocellular hypertrophy were seen in rats and mice. Also, there were significant increases in incidences of centrilobular fatty changes. No carcinogenic activity was observed in rats. In male mice, there was a significant dose-related increase in the incidence of hepatoblastomas. However, the combined incidences of hepatocellular adenoma, carcinoma, and hepatoblastoma were not statistically different from controls. In female mice, there was a significant increase in the combined incidence of hepatocellular adenoma and carcinoma in the 0.25 and 0.5 g/kg groups, but not in the 1.0 g/kg group. In conclusion, the data indicate that chronic administration of kava causes liver toxicity in rats and mice and hepatocellular neoplasms in mice.

PS 2632 USING DNA-BASED MEASUREMENT OF ONCOMUTATION TO UNDERSTAND CARCINOGEN MODE OF ACTION.

Y. Wang, F. Meng, P. B. McKinzie, M. B. Myers and B. L. Parsons. *Division of Genetic and Molecular Toxicology, National Center for Toxicological Research/U.S. FDA, Jefferson, AR.*

Oncogene mutations are considered key events in carcinogenesis and are being developed as quantitative biomarkers of carcinogenic effect. The allele-specific competitive blocker PCR (ACB-PCR) assay is being used to measure levels of oncogenic point mutations in animal and human tissues. This assay measures the ratio of mutant to wild-type allele (mutant fraction) using standards that range from 10^{-1} to 10^{-5}. We will present data from studies employing model carcinogens, including benzo[*a*]pyrene, *N*-hydroxy-2-acetylaminofluorene, aristolochic acid, and azoxymethane which establish four different approaches whereby DNA-based measurement of oncomutation can inform chemical mode of action (MOA). First, induction of two different oncogene mutations, one corresponding to the mutational specificity of the chemical, and the other a frequent spontaneous mutation, has been used to evaluate the extent to which a chemical induces *de novo* oncogene mutation versus amplification of preexisting oncomutation. For example, using ACB-PCR it was shown that both K-*Ras* codon 12 GGT to GAT and GGT to TGT mutations increase as a function of benzo[*a*]pyrene dose in A/J mouse lung. Second, as part of dose-response assessment, oncomutation induction can be integrated with the dose-dependence of other chemical effects. ACB-PCR was used to describe the dose-response relationship between aristolochic acid and induction of H-*Ras* codon 61 CTA mutation in Big Blue rat liver and kidney. Third, oncomutation induction can be integrated with additional endpoints like DNA-adduct formation and/or neutral reporter gene mutation, to establish whether a chemical is carcinogenic through a mutagenic MOA. Fourth, the timing of induction of tumor-associated mutation can be compared to that of other chemical-specific effects, to establish whether induction of mutation is an early biomarker of carcinogenesis.

PS 2633 ANALYSIS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARγ) AGONIST TROGLITAZONE-INDUCED TRANSCRIPTIONAL CHANGES IN MOUSE ENDOTHELIAL CELLS.

S. Kakiuchi-Kiyota[1], L. L. Arnold[1], P. Koza-Taylor[2], S. Suzuki[3] and S. M. Cohen[1]. *[1]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, [2]Pfizer Drug Safety and Development, Groton, CT and [3]Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan.*

A common tumor finding in bioassays of nongenotoxic PPARγ agonists aimed at treating type II diabetes is hemangiosarcoma (HS) in mice, but little is known about their mode of action. We previously showed that troglitazone (TG) increased endothelial cell (EC) proliferation in mice at sarcomagenic doses, and TG directly increased DNA synthesis and inhibited apoptosis in mouse (MFP MVEC) but not in human microvascular ECs. We postulated that TG altered the expression of genes involved in proliferation and apoptosis in mouse ECs, contributing to increased EC proliferation observed in vivo and in HS formation. To identify genes for which expression changed by TG treatment in mouse ECs, MFP MVEC were treated with a reduced concentration (1%) of FBS and 5 μM TG, the concentration of TG that showed the greatest mitogenic effect, for 3 days, and transcriptional profiles were analyzed by microarray analysis. TG significantly altered the expression of 694 genes. The top 10 genes up- or down-regulated in TG-treated MFP MVEC were not detected or predicted as downstream target genes of murine PPARγ signaling. Ingenuity pathway analysis indicated that TG activated the 'Cancer, Cellular Growth and Proliferation, Reproductive Disease' network involving genes implicated in EC proliferation and survival. Among them, insulin-like growth factor 1 (IGF1), which is known to function in tumorigenesis, was most highly up-regulated (log2 transformed ratio: 2.2-fold) in TG-treated MFP MVEC. These results indicate that TG exerts a direct mitogenic and anti-apoptotic effect on mouse ECs by modulating the gene network implicated in EC proliferation and survival, and IGF1 may have an important role in TG-induced HS formation.

PS 2634 RALOXIFENE IS CYTOTOXIC TOWARD VARIOUS ESTROGEN RECEPTOR NEGATIVE CELL LINES.

S. Taurin, B. D. Yadav, K. B. Tran and R. J. Rosengren. *Pharmacology & Toxicology, Univeristy of Otago, Dunedin, New Zealand.*

In recent years, numerous studies have shown that the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene reduce the risk of invasive breast carcinoma. Although long term treatment with tamoxifen increased the risk of endometrial cancer, raloxifene, a second generation SERM, has been shown to be as effective as tamoxifen, while reducing incidence of endometrial carcinoma. However, while SERMs do prevent the development of many estrogen-receptor (ER)-positive breast cancers, few studies suggest a potential effect of these drugs in the prevention of the development of ER-negative breast cancers. Although, the therapeutic efficacy of raloxifene in cancer therapy is thought to arise primarily from its ability to compete with estrogens; raloxifene has been shown to have effects through estrogen receptor (ER)-independent mechanisms. In the present study, we show that raloxifene decreased cell viability of four human ER-negative breast cancer cell lines MDA-MB-231 (EC50 9.5 μM), MDA-MB-468 (EC50 6.8 μM), Hs578t (EC50 6.8 μM) and SkBr3 (EC50 10.6 μM). Cell cycle analysis demonstrated an increased proportion of cells in the G1 phase in all four cell lines. Raloxifene increased apoptosis in MDA-MB-231, MDA-MB-468 and Hs578t by 8 fold and SkBr3 by 6 fold after 48 h. Raloxifene treatment also effected the expression and phosphorylation pattern of proteins involved either in cell proliferation such as NFκB, β-catenin, epidermal growth factor (EGFR), AKT as well as implicated in protein synthesis such as 4EBP-1 or mTOR. Apoptosis was also induced as shown by changes in caspase 3. Furthermore, results from a mouse xenograft tumor model showed that a daily dose of raloxifene (0.85 mg/kg) was sufficient to reduce the tumor size after 10 weeks of treatment. In conclusion, these results provide us with the basis for future studies on the mechanism involved in raloxifene's inhibition of ER-negative tumor growth.

PS 2635 CYTOTOXIC POTENTIAL OF A NOVEL CURCUMIN ANALOG RL-71 IN *IN VITRO* AND *IN VIVO* MODELS OF ESTROGEN RECEPTOR NEGATIVE BREAST CANCER.

B. D. Yadav[1], S. Taurin[1], L. Larsen[2] and R. J. Rosengren[1]. *[1]Pharmacology & Toxicology, Univeristy of Otago, Dunedin, New Zealand and [2]Plant and Food Research Limited, Dunedin, Otago, New Zealand.*

Estrogen receptor negative breast cancer accounts for approximately 30% of all breast cancers. Furthermore, there are limited drug treatments available and patients often suffer from poor prognosis and decreased survival rates. Therefore in

the search of effective drugs for estrogen receptor negative breast cancers, we synthesized and screened new curcumin analogues. Among 28 analogues screened in estrogen receptor negative breast cancer cell lines MDA-MB-231 (ER-/HER2-), MDA-MB-468 (ER-/HER2-) and SKBr3 (ER-/HER2+), RL-71 showed the most potent cytotoxicity. The sulforhodamine B (SRB) assay was used to examine cytotoxicity. Cell cycle distribution and apoptosis induction following treatment with RL-71 was assessed by flow cytometry while protein expression was analyzed by Western blotting. In addition, the in vivo effect of RL-71 on tumor growth was examined in a mouse xenograft model. The results showed that RL-71 had substantially higher cytotoxicity (EC 50 < 0.5μM) compared to curcumin (EC 50 ~ 7 μM). RL-71 arrested MDA-MB-231 and SKBr3 cells in G2/M phase by 162.02 % and 152.94 % respectively and MDA-MB-468 cells in G1 phase by 121.08 % compared to control. Furthermore, RL-71 treatment increased apoptosis in MDA-MB-231, SKBr3 and MDA-MB-468 cells by 10 fold, 6 fold and 4 fold respectively, compared to control. Moreover, RL-71 altered the expression and phosphorylation pattern of variety of proteins which are involved in cell proliferation and apoptosis, such as EGFR, Akt, 4-EBP1, P-38, JNK, NFκB and Caspase 3. However, treatment of a xenograft model with RL-71 at dose of 8.5 mg/kg and 0.85 mg/kg was unable to reduce tumor size compared to control. Thus our findings provide evidence that RL-71 has promising anticancer activity in vitro but it failed to inhibit tumor growth in animal model. The future work in our laboratory will be focused on increasing bioavailability of RL-71 in order to enhance its potency.

PS 2636 RAP80 PLAYS A CRITICAL ROLE IN MAINTAINING GENOMIC STABILITY.

Z. Yin and A. M. Jetten. *LRB, NIEHS, Research Triangle Park, NC .*

The DNA damage response (DDR) coordinates activation of cell cycle checkpoints, apoptosis and DNA repair networks, to ensure accurate repair and genomic integrity. Phosphorylation of the histone H2A variant, referred as γH2AX, is one of the initial signaling events which sense DNA double strand breaks and is required for the subsequent recruitment of many DDR proteins to sites of DNA damage. Upon DNA damage events like ionizing irradiation (IR), ubiquitin interaction motif (UIM)-containing protein RAP80 binds to poly-ubiquitin chain of H2A and γH2AX, and mediates DNA repair events by recruiting DDR mediators and effectors, especially BRCA1. In this study, RAP80 knockout (KO) mice were generated and characterized. In contrast to the embryonic lethal phenotype in BRCA1 KO mice, RAP80 KO mice are viable and there appears to be no major anatomic defect, which suggests RAP80 might be involved in DDR signaling pathways independent of BRCA1. Nevertheless, mouse embryonic fibroblasts (MEFs) from RAP80 KO mice exhibited slower proliferation as well as higher percentage of premature senescence compared to wild type (WT) MEFs. RAP80 KO MEFs also showed increased spontaneous and IR-induced genomic instability, which led to prolonged G2/M cell cycle arrest. There is higher percentage of spontaneous γH2AX positive cells in RAP80 KO MEFs compared to WT MEFs; IR induces more nuclear fragmentation in RAP80 deficient MEFs than WT controls. Loss of RAP80 increased sensitivity to IR both in vivo and in vitro. Preliminary tumorigenesis studies suggested that RAP80 KO mice exhibit an increased susceptibility to the spontaneous development of lymphoma and the development of DMBA-induced mammary gland tumor. Altogether, these data indicate that RAP80 functions as a tumor suppressor gene and deficiency of RAP80 leads to genomic instability and predisposition to cancer.

PS 2637 ACTIN AND VIMENTIN PROTEINS WITH *N*-TERMINAL DELETION DETECTED IN TUMOR BEARING RAT LIVERS INDUCED BY INTRAPORTAL-VEIN INJECTION OF *HA-RAS* TRANSFECTED RAT LIVER CELLS.

Y. Nakamura[1], A. Kominami[1], Y. Tsujimoto[1], E. Park[1], K. Sato[1], C. Chang[2], B. L. Upham[2] and J. E. Trosko[2]. *[1]Food Sciences and Nutritional Health, Kyoto Prefectural University, Kyoto, Japan and [2]National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.*

The F344 rat liver epithelial cell system has been extensively used to study the effects of oncogenes and tumor promoters. Our goal is to develop an *in vivo* model system that can be used to validate and match the *in vitro* results of antitumorigenic studies. The introduction of the tumorigenic *v-Ha-ras* oncogene-transformed rat liver epithelial cells (WB*ras*), which is deficient in gap junctional intercellular communication (GJIC), into F344 rats, induces significant formation of hepatocellular tumors. GJIC plays a major role in maintaining tissue homeostasis. Using this *in vivo* tumor model system, we used 2-dimensional electrophoresis with isoelectric focusing in the first dimension and SDS-PAGE in the second dimension to globally identify proteins that are uniquely expressed in the livers of WB*ras*-treated rats as compared to the sham control. Immunoblotting was used to identify Ras and Connexin43, which were the positive and negative marker proteins, respectively, of the introduced WBras cells. As predicted, immunoblotting indicated that the whole liver of tumor-bearing animals exhibited a decreased level of Connexin43 and an increased level of Ras. Connexin43 and GJIC were expressed and functional in normal liver, but not in the tumor. In addition to these two markers, an additional four proteins exhibited decreased levels and two proteins exhibited increased levels in the livers of tumor bearing animals. *N*-Terminal sequencing analysis was used to identify these proteins which were glucose-regulated protein 78, two isoforms of heat shock protein 60, and the β-chain of ATP synthase for the down regulated proteins, and β-Actin with a 46 amino acid deletion from its *N*-terminus and Vimentin with a 71 amino acid deletion from its *N*-terminus for the up regulated proteins. These data offer potentially new markers of liver tumorigenicity, particularly Vimentin.

PS 2638 SULFORAPHANE INDUCES GLUTATHIONE-*S*-TRANSFERASE ACTIVITY AND DECREASES AFLATOXIN B_1-DNA ADDUCTS IN SPRAGUE-DAWLEY RATS.

J. L. Fiala[1, 2], N. Wiriyachan[3, 4], P. A. Egner[5], J. D. Groopman[5], R. G. Croy[1, 2], P. Navasumrit[3], M. Ruchirawat[3, 4] and J. M. Essigmann[3, 4]. *[1]Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, [2]Chemistry, Massachusetts Institute of Technology, Cambridge, MA, [3]Laboratory of Environmental Toxicology, Chulabhorn Research Institute, Bangkok, Thailand, [4]Faculty of Science, Mahidol University, Bangkok, Thailand and [5]Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.*

Aflatoxin B_1 (AFB_1) contaminated foods increase the risk of liver cancer. Covalent AFB_1-DNA adducts in urine are biomarkers of AFB_1 exposure and have been correlated with increased liver cancer risk. Clinical trials have shown that ingestion of broccoli sprout tea, which is rich in glucoraphanin, can reduce urinary levels of AFB_1 adducts in exposed populations. Sulforaphane (SF; 1-isothiocyanto-4-(methylsulfinyl)-butane), formed from glucoraphanin by myrosinase, is an inducer of Phase II enzymes and is likely responsible for the observed decreased amounts of urinary AFB_1 adducts. We investigated the role of SF in reducing the formation of AFB_1-DNA adducts in the rat, which is highly sensitive to AFB_1 hepatocarcinogenesis. Male Sprague-Dawley rats were treated with SF (3 *x* 125 or 200 mg/kg) or corn oil (*p.o.*). Effects on Phase II metabolism were assessed by measurement of total glutathione-*S*-transferase (GST) activity using the substrate 1-chloro-2,4-dinitrobenzene. SF treatment increased GST activity in rat liver by 1.6 fold ($P<0.05$). We then measured the effect of SF treatment on the AFB_1-DNA adduct levels by administration of [^{3}H]-AFB_1 (25 μg, *i.p.* injection) to rats that had received either 3 *x* 125 mg/kg SF or corn oil. Four hours after AFB_1 administration, liver DNA was isolated and covalently bound [^{3}H]-AFB_1 determined. Levels of AFB_1-DNA adducts were reduced by 49% by SF pretreatment ($P<0.05$). In several species, GSTs have been shown to inactivate the reactive AFB_1-8,9-epoxide preventing formation of covalent adducts. These results indicate that SF induction of GST activity in liver tissue may have a significant role in the chemoprotective activity of broccoli tea.

PS 2639 DOSE RESPONSE CHARACTERIZATION OF DIETARY PHENOBARBITAL-INDUCED GENE EXPRESSION, ENZYME INDUCTION, AND CELL PROLIFERATION IN THE LIVERS OF MALE AND FEMALE MICE.

B. Gollapudi[1], H. Kan[1], A. Wood[1], J. Murray[1], S. Hester[2], A. Goetz[3], R. Currie[4] and D. Geter[1]. *[1]The Dow Chemical Company, Midland, MI, [2]U.S. EPA, Research Triangle Park, NC, [3]Syngenta Crop Protection, Inc., Greensboro, NC and [4]Syngenta Ltd., Bracknell, Berkshire, United Kingdom.*

Phenobarbital (PB) is a prototypical activator of the constitutive androstane receptor (CAR), resulting in liver hypertrophy, hyperplasia, and tumors in mice but only liver hypertrophy in humans. In this study, male and female CD1 mice were exposed to dietary PB at 0, 0.15, 1.5, 15, 75, and 150 mkd for 2 or 7 days. Toxicokinetic investigations showed no deviation from linearity in the dose range used with males having 1.5-fold higher plasma concentration than females at the same dose. Mice exposed to 150 mkd for 2 or 7 days had significantly increased liver weight associated with hepatocellular hypertrophy and increased mitotic figures. Quantitative RT-PCR and enzyme activity (PROD and BROD) highlighted increased CAR activation at concentrations ≥ 15 mkd. Hepatocellular proliferation, quantified by BrdU immunohistochemistry, was increased at ≥ 15 mkd in males and ≥ 1.5 mkd in females, with females showing 1.5-2 fold greater induction in proliferation compared to males. Global gene expression analysis using Affymetrix 430 2.0 arrays revealed little to no pathway alterations at doses ≤ 1.5

mkd. *Cyp2b10* was the strongest indicator of exposure and the overall response indicated increased expression changes of genes whose protein products have functions in cell proliferation and xenobiotic responses. Beyond a predominant metabolic transcriptional response affecting fatty acid and xenobiotic metabolism, other gene expression associated responses were NRF2-mediated oxidative stress, inflammation, numerous cytokine, and apoptotic pathways. Together, these data inform dose, time, and sex effects underlying PB hepatotoxicity in rodents and the mode of action leading to tumor promotion.

PS 2640 OXIDATIVE DNA DAMAGE AND DNA BINDING INDUCED BY 2, 2-BIS (BROMOMETHYL)-1, 3-PROANEDIOL IN HUMAN BLADDER CELLS: POSSIBLE DUAL MECHANISMS IMPLICATED IN ITS CARCINOGENICITY.

W. Kong, R. K. Kuester, A. Gallegos and I. Sipes. *Pharmacology, University of Arizona, Tucson, AZ.*

2, 2-Bis (bromomethyl)-1, 3-propanediol (BMP) is a flame retardant found in urethane foams and polyester resins. In a two year dietary study, BMP caused neoplastic lesions at multiple sites including the urinary bladder of rats and mice. The mechanism of its carcinogenic effect is unknown. In this in vitro study, using SV-40 immortalized human uroepithelial cells (UROsta), binding of BMP to DNA and BMP induced DNA damage were investigated. DNA binding levels were measured at various time points between 1 and 24 h of incubation of UROsta cells with 14C-BMP (10 μM). The effects of time (1-24 h) and concentration (5-100 μM) on DNA strand breaks were assessed via the standard alkaline comet assay. Binding of total BMP to DNA increased from 3 to 38 pmol per mg DNA over the incubation time (resistant to RNAase and proteinase digestion and phenol-chloroform extraction). BMP significantly increased the extent of DNA strand breaks at 1 and 3h of incubation. However, this DNA damage returned to basal level after 6h. Because the induction of strand breaks preceded the maximum binding, a potential role for BMP associated oxidative damage to DNA was investigated. Cell lysates were incubated with a lesion specific endonuclease, human 8-hydroxyguanine DNA-glycosylase (hOGG1) before the alkaline comet assay to reveal oxidized guanine as strand breaks. Also, intracellular reactive oxygen species (ROS) were measured by a ROS sensing fluorescence probe, H2DCFDA. An increase in strand beaks upon incubation with hOGG1 was observed in the modified comet assay at 1h following BMP exposure. Furthermore, evidence for BMP (10, 25,100 μM) associated oxidative stress was shown as an elevation of intracellular ROS generation within 1h. These results demonstrate that in UROsta cells BMP binds to DNA and also induces DNA strand breaks, some of which result from oxidative DNA lesions. Both of these events, DNA binding and oxidative DNA damage may, in part, contribute to BMP carcinogenicity.

PS 2641 TRICHLOROETHYLENE- AND TETRACHLOROETHYLENE-INDUCED RENAL CARCINOGENESIS: INSIGHTS FROM HUMAN, ANIMAL BIOASSAY, AND MECHANISTIC STUDIES.

M. R. Gwinn, K. Z. Guyton, J. Jinot, C. S. Scott, K. A. Hogan and W. A. Chiu. *U.S. EPA, Washington, DC.*

A review was conducted of epidemiologic, animal bioassay and mechanistic data for insights regarding the effects of trichloroethylene (TCE) and tetrachloroethylene (PCE) on kidney toxicity and carcinogenicity. Numerous independent epidemiologic studies have associated TCE exposure with elevated kidney cancer risk and statistically significant exposure-response trends, and the consistency of the findings suggests a causal relationship between TCE exposure and kidney cancer. For PCE, the epidemiologic evidence of kidney cancer is more limited, with most studies examining job or occupational title. The rodent bioassay database includes multiple lifetime studies reporting small increases in renal tubule tumors, including very rare malignant adenocarcinomas, primarily in male rats following inhalation or oral exposure to TCE or PCE. Renal toxicity but not cancer is reported in mice. Results of in vivo and in vitro studies support a prominent role for metabolites derived from the GSH conjugation pathway of TCE or PCE metabolism in renal toxicity and tumorigenicity. This includes the demonstrated in vitro mutagenicity of the GSH-derived PCE and TCE metabolites, or of both parent compounds under conditions that would generate these metabolites, and evidence of their generation in the kidney following PCE or TCE exposure. The role of GSH conjugates is further supported by recent epidemiologic evidence of elevated kidney cancer risk in TCE-exposed subjects with at least one intact GSTT1 allele but not among subjects with two deleted alleles. While the mechanisms of PCE- and TCE-induced renal carcinogenesis have not been fully elucidated, this review of the available evidence supports the contribution of these mutagenic metabolites to the development of the kidney tumors for either compound. Some evidence supports a role of cytotoxicity leading to compensatory cellular proliferation in renal carcinogenesis, either together with or independent of mutagenicity. This abstract does not represent EPA policy.

PS 2642 COMPARISON OF AFLATOXIN-DNA ADDUCT LEVELS IN MALE AND FEMALE INFANT B6C3F1 MICE.

L. L. Woo[1], P. A. Egner[2], C. Belanger[1], R. Wattanawaraporn[1], L. Trudel[1], G. N. Wogan[1, 3], J. D. Groopman[2], R. G. Croy[1, 3] and J. M. Essigmann[1, 3]. [1]*Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA,* [2]*Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD and* [3]*Chemistry, Massachusetts Institute of Technology, Cambridge, MA.*

The infant mouse model is useful for identification of developmental factors responsible for the age and gender differences in sensitivity towards AFB_1 and other carcinogens. Male infant B6C3F1 mice are highly sensitive to the hepatocarcinogenic effects of aflatoxin B_1 (AFB_1), while infant females are refractory. A multi-dose regimen of AFB_1 beginning at 4 days of age produces hepatocellular carcinoma in >90% of males at 18 months; no tumors are observed in females at that time point. Adult mice of both genders, unlike infant males, are resistant to AFB_1's carcinogenic effect. AFB_1 is metabolized to the 8,9-epoxide which covalently modifies DNA, forming mutagenic AFB_1-N7-Gua and AFB_1-FAPY adducts. We investigated whether male and female infant mice are at similar risk of genetic damage from AFB_1. AFB_1 adducts in liver DNA were measured 24 h following administration of either a single 6 μg/g dose on day 4 or as 3 x 2 μg/g doses administered on days 4, 7 and 10 of age. AFB_1 adducts were released from isolated DNA by acid hydrolysis and quantified by HPLC-MS/MS using AFB_1-$^{15}N_5$-Gua internal standards. Following a single 6 μg/g AFB_1 dose, 3.4± 1.7 and 2.8±0.90 AFB_1-N7-Gua adducts/10^6 DNA bases were found in male and female mouse livers, respectively. Twenty-four h after the final 2 μg/g dose, levels of AFB_1-N7-Gua were much lower at 0.37±0.21 adducts/10^6 bases in males and 0.40±0.20 adducts/10^6 bases in females. AFB_1-FAPY adducts were at higher levels in the multi-dose animals; 3.6 ±1.9 AFB_1-FAPY adducts/10^6 bases in males and 2.5±1.2 AFB_1-FAPY adducts/10^6 bases in females. These results indicate that similar amounts of AFB_1-DNA adducts are formed in male and female infant B6C3F1 mice. Thus, both genders appear to be at a similar risk of mutations from covalent AFB_1 adducts formed in liver DNA.

PS 2643 NO LUNG TOXICITY FROM STYRENE IN CYP2F2 KNOCKOUT MICE.

G. Cruzan[1], J. S. Bus[2], X. Ding[3], J. A. Hotchkiss[2], J. R. Harkema[4] and R. Gingell[5]. [1]*ToxWorks, Bridgeton, NJ,* [2]*Toxicology and Environmental Research and Consulting, Dow Chemical Company, Midland, MI,* [3]*New York State Department of Health, Wadsworth Center for Labs. and Research, State University of New York at Albany, Albany, NY,* [4]*Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI and* [5]*Environmental and Product Health, Shell Oil Company, Houston, TX.*

Styrene induces lung tumors in mice, but not in rats. We hypothesized that the mouse lung tumors were mediated by mouse specific CYP2F2 generated cytotoxic, ring-oxidized metabolite(s) producing repeated localized injury to Clara cells, the site of CYP2F2 metabolism and the tumorigenic response. This hypothesis was consistent with previous data indicating that styrene lung toxicity was not attenuated in CYP2E1 knockout mice (2E1 produces styrene-7,8-oxide). To test our hypothesis we developed CYP2F2-/- (KO) mice on a C57BL/6 background. The 2F2-KO mouse was produced by replacing the third coding exon with a neomycin resistance gene followed by homologous recombination in C57BL/6-derived Bruce4 mouse embryonic stem cells. Male WT and KO mice received 0 or 400 mg/kg/day styrene in corn oil by gavage for 5 days (n=7/group). WT mice treated with 400 mg/kg styrene displayed mild atrophy of the bronchiolar epithelium due to necrosis and exfoliation of Clara cells concurrent with areas of epithelial regeneration. The cumulative BrdU-labeling of S-phase cells (LI) was markedly increased in both proximal (46.8±7.9%) and terminal bronchioles (62.4± 2.7%) compared to WT control mice (2.2±0.5% and 4.4±0.9%, respectively). No evidence of Clara cell toxicity was observed in the pulmonary airways of 400 mg/kg-treated KO mice. The cumulative LI was not increased in proximal (1.1±0.3%) or terminal bronchioles (2.2±0.7%) compared to KO control mice (3.2±0.6% and 2.0±0.7%, respectively). This study clearly demonstrates that styrene toxicity in the mouse lung is dependent on metabolism by CYP2F2, and that the mouse tumor response may not be relevant to human risk in that human lung expresses CYP2F1, an isoform not suspected of catalyzing significant styrene metabolism.

PS 2644 COVALENT BINDING OF ^{14}C 2-METHOXY-4-NITROANILINE IN MALE SPRAGUE-DAWLEY RATS.

R. Snyder[1], S. Waidyanatha[2], I. Surh[2], T. Banks[1], P. Patel[1], S. Black[1] and T. Fennell[1]. *[1]RTI International, Research Triangle Park, NC and [2]National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.*

2-Methoxy-4-nitroaniline (MNA) is used in dyeing textiles, as a dye in the printing industry, and as an intermediate in the synthesis of azo dyes that have applications in tattoo inks, emulsion paints, and toy enamels. MNA has structural similarity to the known carcinogens, 2-methoxy-5-nitroaniline (5-nitro-o-anisidine), o-anisidine and 2,4-diaminoanisole. The objective of this study was to evaluate the ability of MNA-derived metabolites to bind covalently to tissue macromolecules in rats. [Ring-^{14}C] MNA was administered at 150 mg/kg b. wt. to male Sprague Dawley rats (approximately 220 μCi/rat) by gavage. After 24 h, animals were euthanized and blood and selected tissues were collected and analyzed for total radioactivity in tissues, radioactivity bound to tissues and to DNA. In tissues, the total radioactivity was highest in liver (0.31 ± 0.04 % of the dose) and kidney (0.053 ± 0.021 %). However, the concentration of radioactivity was highest in kidney and cecum (91 ± 4 and 22 ± 1 μg equiv/g tissue, respectively). Concentrations were similar in ileum, jejunum, and large intestine (approximately 16 μg equiv/g). Radioactivity bound determined by exhaustive extraction was highest in liver (43% of the total in tissue) followed by kidney (13 % of the total in tissue), cecum (12 %) stomach (9 %), and large intestine (9 %). Binding was low in ileum, jejunum, and duodenum (1-2% bound). In red blood cells, 27% of the activity was bound compared with 3% in plasma. Radioactivity associated with DNA (pmol equiv/mg DNA) was highest in the cecum (160 ± 27 pmol/mg) followed by large intestine (13 ± 2 pmol/mg), and was not significantly above background in other tissues. These studies suggest that MNA is metabolized to reactive species that are capable of binding to macromolecules including DNA. The nature of the metabolites of MNA is under investigation in companion studies.

PS 2645 DIFFERENTIAL MODULATION OF CANCER-RELATED MOLECULAR NETWORKS IN HUMAN AND RAT URINARY BLADDER CELLS EXPOSED TO TRIVALENT ARSENICALS.

K. Bailey[1], K. Wallace[2], S. Thai[2], D. C. Wolf[2], S. W. Edwards[2] and R. C. Fry[1]. *[1]Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC and [2]U.S. EPA, Research Triangle Park, NC.*

Arsenic (As) is classified as a known human carcinogen with primary targets of urinary bladder (UB), skin and lung. The most prevalent source of As exposure in humans is drinking water contaminated with inorganic As (iAs). The mode of action (MOA) of As carcinogenesis in target cells is largely undefined, including which arsenical(s) elicit a carcinogenic response. Two urinary metabolites of iAs, monomethylarsonous acid and dimethylarsinous acid (MMAIII and DMAIII, respectively), are attractive candidates as UB carcinogens. We used a transcriptomics approach to examine the altered molecular pathways and networks in human and rat UB cells after exposure to individual arsenicals to investigate the MOA of As-driven UB carcinogenesis. UROtsa (human) and MYP3 (rat) cells were exposed to relatively non-cytotoxic (>75% cell viability), environmentally-relevant concentrations (1 μM) of iAsIII, MMAIII, and DMAIII for 24 h. Differentially expressed genes were determined for each treatment group relative to controls and analyzed for statistically significant biological functions, molecular networks and canonical pathways. Each treatment group elicited a distinct transcriptional profile with few shared genes in top networks or canonical pathways, although lipid metabolism was a function associated with all top networks. Distinct from the other groups, MMAIII exposure in UROtsa cells generated changes in several top molecular networks that are consistent with pathways suspected to play key roles in human UB carcinogenesis, suggesting that common factors may drive UB carcinogenesis in humans and that they may be distinct from those in rats. [This abstract does not necessarily reflect EPA policy.]

PS 2646 THE EFFECTS OF ORAL TREATMENT WITH TRANSFLUTHRIN ON THE BLADDER EPITHELIUM OF RATS AND MICE AND ITS METABOLITE, TETRAFLUOROBENZOIC ACID ON UROTHELIAL CELLS *IN VITRO*.

S. M. Cohen[1], L. Arnold[1], S. Lautraite[2], L. Sheets[2], S. Wason[2], B. Stahl[2], D. Eigenberg[3], K. L. Pennington[1], S. Kakiuchi-Kiyota[1] and M. Yokohira[1]. *[1]University of Nebraska Medical Center, Omaha, NE, [2]Bayer AG, Bayer CropScience, Monheim, Germany and [3]Xenometrics, Stilwell, KS.*

Transfluthrin, a pyrethroid insecticide, induced urinary bladder tumors in rats but not in mice in 2-year bioassays. The mechanism was investigated via an in vivo study with transfluthrin in rats and mice and in vitro studies to examine the effects of its major metabolite tetrafluorobenzoic acid (TFBA) on rat (MYP3) and human (1T1) urothelial cell lines. For the in vivo study, rats were fed diet containing 0, 2000 or 5000 ppm transfluthrin for 4 weeks, 0 or 2000 ppm for 13 weeks, or co-adminstered 1.25% NH4Cl with 0 or 5000 ppm transfluthrin to evaluate the effects of acidification of the urine. Mice were treated with 0 or 1000 ppm transfluthrin for 4 weeks. After 4 weeks, there was no evidence of hyperplasia or increased bromodeoxyuridine labeling index in any treatment group. After 13 weeks treatment with 2000 ppm transfluthrin, cytotoxicity and necrosis of the urothelial superficial layer were detected in the rat bladder by scanning electron microscopy. The urinary concentration of TFBA in rats fed 2000 ppm transfluthrin was 2.94±0.67 mM. The LC50 of TFBA was 2.25 mM for MYP3 cells and 2.43 mM for 1T1 cells. These studies support cytotoxicity and regenerative proliferation as the mechanism for induction of bladder tumors with high oral doses of transfluthrin due to metabolism of transfluthrin to the weakly cytotoxic TFBA that is excreted at high concentrations in the urine of rats administered high doses of transfluthrin (≥2000 ppm) for an extended period.

PS 2647 NEUROBEHAVIORAL ALTERATIONS IN CARG-BOX BINDING FACTOR-A KNOCK-OUT MICE.

J. T. Barrett[1, 2], J. R. Richardson[2], X. Zhang[3], M. Fang[2], K. R. Reuhl[2, 4] and H. Zarbl[2]. *[1]GSBS, UMDNJ, Piscataway, NJ, [2]EOHSI, RWJMS, UMDNJ, Piscataway, NJ, [3]University of Washington, Seattle, WA and [4]Rutgers, Piscataway, NJ.*

CArG-box binding factor A (CBF-A) is a member of the heterogeneous nuclear ribonucleoproteins (hnRNPs) family of RNA-binding proteins that are involved in a variety of cellular functions. We previously showed that deregulation of CBF-A/hnRNP A/B is involved in transcriptional regulation of the HA-ras promoter and contributes to mammary carcinogenesis. Recently, we have developed CBF-A knock-out mouse models to further study the functions of this hnRNP protein. In addition to its role in mammary carcinogenesis, CBF-A is also expressed in the brain and is involved in regulation of immediate early gene expression. Furthermore, a single nucleotide polymorphism (SNP) in the arginine vasopressin gene promoter that reduced CBF-A binding was associated with alterations in anxiety behavior. To directly determine the role of CBF-A in neurobehavior, we assessed open-field locomotor function and anxiety behavior in an elevated plus maze for wild type, heterozygous, and CBF-A null mice. For locomotor activity, mice were allowed to habituate to the open-field for 30 min and ambulatory distance and vertical counts were calculated over the next 30 min. For the elevated plus maze, mice were allowed to freely explore the maze for 5 min, and percent time spent in the open arms was determined. In both tasks, there were no significant differences between wild-type and mice heterozygous for CBF-A. However, ambulatory distance traveled was increased by 89% in CBF-A null mice compared to wild-type. CBF-A null mice also demonstrated a 230% increase in vertical activity. In the elevated plus maze, CBF-A null mice spent 319% more time in the open arms of the maze compared to wild-type, suggesting that loss of CBF-A results in a low-anxiety phenotype. Taken in concert, these data suggest that CBF-A plays an important role in neurobehavioral function. Supported by R01ES015991 and P30ES005022

PS 2648 INFLUENCE OF BENZO(A)PYRENE (BAP) BIOTRANSFORMATION ON BAP-INDUCED CYTOTOXICITY IN HT-29 HUMAN COLON CANCER CELLS.

J. N. Myers and A. Ramesh. *Biochemistry & Cancer Biology, Meharry Medical College, Nashville, TN.*

The majority of sporadic cancer deaths are attributed to exposure and intake of environmental toxicants. Benzo(a)pyrene (BaP) is one such ubiquitous environmental toxicant. Being a combustion byproduct, high levels of BaP have been detected in

barbecued pork and beef meat, contributing to a substantial dietary intake of this toxicant. Studies conducted in our lab have shown that BaP contributes to colon cancer development in animal models. Through metabolic activation, BaP is biotransformed into reactive metabolites that bind with DNA, cause DNA damage and associated changes in cell cycle. The purpose of this study was to determine the concentrations of BaP that are most toxic to HT-29 human colon cells. Cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% FBS and antibiotics (penicillin/streptomycin). After revival of cells and growing them to confluence, cells were synchronized overnight by serum starvation method. The HT-29 cells were exposed to 1, 5, 10, 25, 50, and 100μM BaP over the course of 10 days and then counted using a Coulter Counter. Based on a concentration – response study 5, 10, and 25μM BaP in DMSO (vehicle for BaP; 0.01%); 4 days after exposure were selected as optimal concentrations to analyze the effect of BaP on cell growth, viability, biochemical, and molecular endpoints. The expression of both Phase I and II drug metabolizing enzymes were analyzed by western blot and RT-PCR. Analysis of cell cycle via FACS showed a distinct inhibition of the S and G2-phase in cells treated with increasing concentrations of BaP. An increase in CYP 1A1 and 1B1 expression also occurred in a BaP concentration-dependent manner. On the other hand, not much difference was seen in Phase II metabolizing enzyme expression levels regardless of BaP concentrations. Our results suggest that BaP cytotoxicity is controlled by metabolic activation. This study was funded by the NIH grants 5T32HL007735-12, 1RO1CA142845-01A1, 1RO3CA130112-01, 1S11ES014156-01A1 and Southern Regional Education Board.

PS 2649 EFFECTS OF SUBCHRONIC SODIUM ARSENITE ON MALIGNANT TRANSFORMATION MARKERS IN MCF-7 CELLS.

A. D. Rios-Perez[1], R. Ruiz-Ramos[1], L. T. Lopez-Carrillo[2] and M. E. Cebrian[1]. *[1]Toxicology, CINVESTAV, Mexico City, DF, Mexico and [2]CISP, INSP, Cuernavaca, Morelos, Mexico.*

Inorganic arsenic (iAs) is a well-known human carcinogen, however its mechanisms still require further investigation. We have reported that arsenite induced cell proliferation, increased S-phase cell recruitment, and ROS generation in MCF-7 cells, promoting genomic instability by damaging DNA and inducing oncogene expression and cell cycle regulatory proteins. We also showed induced protein levels of metallothioneins 1 and 2, PCNA and p53 in the presence of decreased p21 levels, all of which contributed to explain cell cycle disruption. Regarding other potential mechanisms of As-induced cancer, S100 proteins are small molecules of the EF hand type, which have been functionally linked with cell growth, motility, invasiveness, matrix metalloprotease (MMP) overexpression and alterations in p53 activity. Furthermore, alterations in S100 expression have been associated with gastric, colorectal, lung and breast cancer, and have been proposed as staging and prognostic tools. The aim of this study was to evaluate the effect of sodium arsenite over S100A4/7 expression and its relationship with MMP9/13. Cells exposed to arsenic (0-10 uM during 0-8 h) showed increased S phase recruitment, as evaluated by FACS. In addition, treated cells also showed S100A4, S100A7 gene overexpression, and increased protein levels, as analyzed by PCR-RT and Western Blot. In addition, cells subchronically exposed to iAs (0.25 uM; 8 weeks) showed S100A7, MMP9 and MMP13, gene overexpression. Only S100A7 was down regulated at 1 uM, while S100A4 was downregulated at 0.25 and 1 uM. These findings suggest that arsenic is able to deregulate S100A7 and MMP9/13 gene expression and their potential relationship. Further studies are required on the mechanisms underlying a potential malignant transformation induced by As in MCF-7.

PS 2650 DOES HELICOBACTER PYLORI PARTICIPATE IN T HELPER-1 MEDIATED IMMUNE ACTIVATION IN COLORECTAL CANCER PATIENTS?

B. A. Engin[1], A. Karakaya[1] and A. Engin[2]. *[1]Department of Toxicology, Gazi University, Faculty of Pharmacy, Ankara, Turkey and [2]Department of General Surgery, Gazi University, Faculty of Medicine, Ankara, Turkey.*

Controversial findings are present on the association between increased risk of colorectal carcinoma and chronic infection. Colonization of Helicobacter pylori (H.pylori) increases risk of the development of colorectal cancer by 2.2 fold. However, it is not clear whether the H.pylori stimulate the cell-mediated immunity or tryptophan degredation pathway of cancer patients. The aim of this study was to evaluate the cellular immune activation by measuring serum neopterin and estimate T-cell responsiveness by calculation of kynurenine to tryptophan ratio. Thus, in this study, ninety seven patients (19 were H.pylori positive and 78 were H.pylori negative) with colorectal cancer diagnosis conducted as cancer-group. One hundred eight cancer-free individuals (37 were H.pylori positive and 71 were H.pylori negative) were constituted the control group. H.pylori IgG seropositivity was detected by enzyme linked immunosorbent assay (ELISA). Serum tryptophan and kynurenin were measured by high performance liquid chromatography. Indolamine-2,3-dioxygenase (IDO) activity was calculated by kynurenine/tryptophan. Serum neopterin was detected by ELISA. The measured serum parameters of H.pylori negative control group compared to the positive control group were not significantly different ($p>0.05$). There was an increased cellular immune activation in H.pylori negative colorectal cancer patients compared to the H.pylori negative controls, as well as in H.pylori positive cancer patients compared to H.pylori positive controls (both, $p<0.05$). As a conclusion, presence of H.pylori colonization in colorectal cancer do not soley affect serum IDO activity and cell mediated immunity indicated by the increased serum neopterin levels ($p>0,05$). However, colorectal cancer causes the stimulation of macrophages and increases the kynurenine synthesis independent of the H.pylori existence.

PS 2651 TOXICOGENOMIC PROFILING OF FORMALDEHYDE-EXPOSED NORMAL AND TRANSFORMED HUMAN ORAL KERATINOCYTES.

R. Ceder[1], M. Merne[1], J. Nilsson[1], C. Staab[2], J. Höög[2], C. M. Thompson[3] and R. C. Grafström[1, 4]. *[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, [3]ToxStrategies, Inc., Katy, TX and [4]Medical Biotechnology, VTT Technical Research Centre of Finland, Turku, Finland.*

Toxicogenomic assessments in vitro might serve to generate novel toxicity biomarkers and support the replacement of animals in toxicity testing. Toxicity of formaldehyde (FA), a classified human carcinogen, was studied in a cell culture model for cancer development that included normal (NOK), immortalized (SVpgC2a) and malignant (SqCC/Y1) cells. Repeated 1h FA exposures of SVpgC2a induced transformation as indicated from cell crisis and the generation of a novel line (SVpgC3a) with altered morphology (e.g., multifocal, soft agar growth, but non-tumorigenic in athymic nude mice). Assessment of cytotoxicity (MMT method) and genotoxicity (DNA protein crosslinks) indicated similar initial damage levels in all lines whereas the longer-term consequences differed (cell number and cloning assessments indicated a sensitivity order of NOK>SVpgC2a>SVpgC3a≈SqCC/Y1). FA induced cell death primarily by terminal differentiation in NOK whereas differently SVpgC2a, SVpgC3a and SqCC/Y1 died by other means e.g., apoptosis. Interestingly, over a dose range, the highest levels of micronuclei in SVpgC2a were observed at the FA concentration that induced cell transformation. Proteomics and transcriptomics profiling of SVpgC3a versus SVpgC2a showed that cell transformation coupled with multiple changes in single genes, molecular networks and gene ontologies. Likewise, transcript profiling of the respective cell lines for up to 48h following 1h FA exposure indicated multiple genomic changes, some of which overlapped to results from cancer-inducing protocols in vivo. In conclusion, the sensitivity to formaldehyde toxicity might differ between stages in cancer development, and specific gene expression changes couple to the respective phenotypes. Generation of the SVpgC3a line extended the current cancer model of normal, immortal and malignant cells.

PS 2652 A GENOME-WIDE CELL SIGNALING RNA-INTERFERENCE SCREEN TO IDENTIFY NOVEL REGULATORS OF THE DNA DAMAGE RESPONSE.

B. van de Water[1], J. Carreras[1], L. von Stechow[1], R. Sidappa[1], A. Pines[2], J. Olsen[3], H. Vrieling[2], L. Mullenders[2] and E. Danen[1]. *[1]Division of Toxicology, Leiden University, Leiden, Netherlands, [2]Department of Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands and [3]Panum Institute, University of Copenhagen, Copenhagen, Denmark.*

The DNA damage response (DDR) involves a complex of signaling events that participate in lesion recognition, cell cycle arrest, damage repair, and ultimately cell cycle re-entry or, if repair fails, initiation of cell death. The DDR is critical for maintenance of genomic integrity and its failure is associated cancer. In a highly simplified model DNA-damage causes activation of the sensor kinases (e.g. ATM, ATR, DNA-PK) that activate p53 and the checkpoint kinases (i.e. Chk1, Chk2), which in turn regulate effector pathways that the above programs. In reality, the process is much more complex with many additional regulators, extensive cross-talk, and multiple positive and negative feedback loops. We have taken a "systems approach" to map the DDR signal transduction network in more detail. For this we used embryonic stem cells treated with the DNA cross-linking agent cisplatin and systematically determined the differential changes at the transcriptome and (phospho)-proteome level. These findings were combined with high-throughput RNA-interference-based functional genomics approaching using genome wide siRNA li-

braries targeting all transcription factors, protein kinases and phosophatases and (de)-ubiquitinases. The siRNA screens resulted in novel players that control the DDR response. We have integrated these functional critical components of the DDR with the phospho-proteomics and transcriptomics data to generate a comprehensive understanding of the DDR signaling network. This information not only increases the level of understanding of the DDR program but also may identify improved mechanism-based biomarkers for genotoxicity. In addition, these novel DDR regulators may be future candidate drug targets for either increasing sensitivity of otherwise drug resistant tumor cells or protect normal cells from DNA-damaging anticancer drugs. Sponsored by the Netherlands Toxicogenomics Center

PS 2653 GLUCOSE ENHANCES MENADIONE MEDIATED HYDROGEN PEROXIDE FORMATION IN BETA CELLS.

M. A. Palo[1], T. L. Womack[1], E. Heart[2] and J. P. Gray[1, 2]. [1]*Science - Chemistry, United States Coast Guard Academy, New London, CT and* [2]*Biocurrents Research Center, Marine Biological Laboratory, Woods Hole, MA.*

During tumor development, cancer cells undergo dramatic changes in cellular metabolism, including decreased mitochondrial respiration and enhanced glycolysis. They also express elevated levels of glucose transporters to support changes in glycolysis. Redox cycling chemicals such as menadione are enzymatically reduced by cellular reductases and chemically reduce oxygen, resulting in the formation of reactive oxygen species including superoxide anion and hydrogen peroxide. In the current work, we hypothesized that redox cycling in cancer cells is glucose-dependent. However, because cancer cells constitutively express multiple glucose transporters, we utilized the beta cell model INS-1 832/13, which expresses a unique glucose transporter (GLUT2) that rapidly equilibrates glucose. In these cells, menadione was found to stimulate formation of ROS; this was concentration-dependent in the range of 0.1-10 μM. To test the importance of glucose for chemical redox cycling, cells were treated with 10 μM menadione and increasing concentrations of glucose (2-16 mM). We found that the rate of hydrogen peroxide formation increased five-fold over this concentration range. Concentrations of glucose inducing maximal release of hydrogen peroxide directly correlated with maximal insulin release from these cells. The non-metabolizable sugar derivative 2-deoxy-D-glucose did not support increases in hydrogen peroxide formation suggesting that glucose metabolism is required for the generation of ROS. Taken together, these data suggest that INS-1 832/13 cells are a useful model for investigating the interaction of glucose metabolism and redox cycling. The glucose-dependent formation of reducing agents supports chemical redox cycling, and the overexpression of glucose transporters in cancer cells might be exploited for chemotherapeutic design.

PS 2654 THE DURATION OF THE EVALUATION PERIOD OF THE POSITIVE CONTROL GROUP FOR RASH2 SHORT-TERM CARCINOGENICITY STUDIES.

S. A. Shah, M. A. Paranjpe and E. A. Zahalka. *Mammalian Toxicology, BioReliance Corporation, Rockville, MD.*

Short-term (26-week) transgenic mouse studies have been accepted by the regulatory agencies as an alternative model in place of the conventional 2-year mouse carcinogenicity models, for the last 14 years. In these studies a positive control group is used to demonstrate the validity of the test system's susceptibility to carcinogenicity and to ensure the use of the proper transgenic mouse strain. The duration period of the observations following treatment is currently set at 17 weeks in our study protocols. At the end of this period mice are evaluated macro- and microscopically. Shortening this evaluation period without impacting the objectives of the positive control group in these studies is of considerable interest, especially from an animal welfare perspective. Accordingly, we have designed a study to address this issue using the RasH2 model since it is currently the most widely used model in the industry. The study included five groups treated with Urethane (1000 mg/kg), and one control group (saline). Each group consisted of 10mice/sex. Mice were administered a total of 3 intraperitoneal injections of Urethane (1000 mg/kg) on study days 1, 3, and 5. Animals were evaluated macroscopically on weeks 8, 10, 12, 14 or 16 following treatment. All animals treated with Urethane responded positively to the treatment, as was evident by post-dose clinical observations (i.e. ataxia, decreased motor activity, prostrate positioning, labored/rapid/shallow breathing). Macroscopic observations at necropsy revealed that control animals were tumor free at 16 weeks, while urethane-treated mice showed tumors in the lungs and spleen as early as 10 weeks post treatment. At 8 weeks post treatment only the lung responded positively. In conclusion, we have demonstrated that it may not be necessary to retain the positive control animals on study beyond week 10 for scoring tumors. Ongoing microscopic evaluations will enable confirmation of these findings.

PS 2655 CYTOTOXICITY OF ARSENICALS ON HUMAN BRONCHIAL EPITHELIAL CELLS *IN VITRO.*

L. Arnold, S. Kakiuchi-Kiyota, K. L. Pennington and S. M. Cohen. *University of Nebraska Medical Center, Omaha, NE.*

Inorganic arsenic [arsenate (As^V) and arsenite (As^{III})] is a known human carcinogen, inducing tumors of the skin, urinary bladder and lung in individuals exposed to high concentrations, primarily through drinking water. The mechanism by which inorganic arsenic induces tumors is being studied. *In vivo*, we have shown that inorganic arsenic induces cytotoxicity with increased cell proliferation and regenerative hyperplasia in the urinary bladder of rats and mice. *In vitro*, the trivalent arsenicals As^{III}, monomethylarsenous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) are highly cytotoxic to human and rat urothelial cells compared to the corresponding pentavalent forms. In addition, we have shown that the pentavalent thio-metabolite, dimethylmonothioarsinic acid ($DMMTA^V$) is cytotoxic to rat and human urothelial cells at concentrations similar to those of the trivalent arsenicals. In this study, we examined the cytotoxicities of various arsenicals on human bronchial epithelial cells (HBEC) isolated from bronchial brush biopsies. The trivalent arsenicals and $DMMTA^V$ were cytotoxic at micromolar concentrations (As^{III}-5.8 μM, MMA^{III}-1.0 μM, DMA^{III}-1.4 μM, $DMMTA^V$-5.5 μM). As^V was also cytotoxic at micromolar concentrations, but at a much higher level compared to As^{III} (As^V-46.5 μM). The pentavalent organic arsenicals monomethylarsonic acid (MMA^V) and dimethylarsinic acid (DMA^V) were cytotoxic at millimolar concentrations (MMA^V-6.1 mM, DMA^V-1.2 mM). These results show that arsenicals are cytotoxic to human bronchial epithelial cells at concentrations similar to those that induce cytotoxicity in human and rat urothelial cells.

PS 2656 FORMATION OF TAMOXIFEN (TAM)-DNA ADDUCTS IN MONKEYS AND HUMANS.

E. Hernandez Ramon[1], K. John[1], R. A. Woodward[2] and M. C. Poirier[1]. [1]*NCI/NIH, Bethesda, MD and* [2]*NICHD/NIH, Poolesville, MD.*

Tamoxifen (TAM) is currently given to thousands of women and is a highly-successful adjuvant therapy for estrogen-receptor-positive breast cancer. However women receiving TAM, also have an increased risk of endometrial and myometrial cancer, which may be due to genotoxicity or may result from receptor-related mechanisms. Controversy has surrounded the issue of whether or not TAM-DNA adducts form in human tissues. To address this, we have examined TAM-DNA adduct formation in multiple tissues of Erythrocebus patas (patas) monkeys given oral TAM dosing for 3 months, and in endometrial and myometrial tissues of human cancer patients receiving TAM therapy. Adducts were determined by TAM-DNA chemiluminescence immunoassay (CIA) using an antiserum elicited against DNA modified with (E)-α-(deoxy-guanosin-N^2-yl)-tamoxifen (dG-TAM). Oral dosing with TAM (1.7 mg TAM/kg/day) was given to 3 adult (22 yrs) patas females for 3 months. Two unexposed patas of similar age were used as controls. DNA, extracted from several organs, was analyzed by TAM-DNA CIA. The highest TAM-DNA adduct levels were found in liver (40.3±3.5 adducts/10^8 nucleotides, mean ± SE). Measurable levels were also found in uterus (35.1±11.7 adducts/10^8 nucleotides), brain cerebellum (10.1±2.9 adducts/10^8 nucleotides) and esophagus (13±3 adducts/10^8 nucleotides). However, no adducts were detectable in brain cortex, ovary and kidney. Four samples of endometrial tumor from patients receiving TAM therapy had values of 10-16.5 adducts/10^8 nucleotides, while no TAM-DNA adducts were detected in 8 patients not receiving TAM therapy. These studies are being expanded to include determination of TAM-DNA adducts by HPLC-MS/MS and TAM-DNA immunohistochemistry in human and primate. These data show that TAM-DNA adducts can be formed in primate reproductive organ tissues as well as human endometrial tumors, suggesting that TAM-DNA adduct formation may play a role in the development of human endometrial cancer.

PS 2657 INVOLVEMENT OF CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) IN THE PROCESS OF LIVER HYPERTROPHY AND HEPATOCARCINOGENESIS-INDUCED BY CYP2B-INDUCING NON-GENOTOXIC HEPATOCARCINOGENS IN MICE (2).

K. Inoue[1], M. Yoshida[1], Y. Sakamoto[1], M. Takahashi[1], Y. Taketa[1], S. Hayashi[1], S. Ozawa[2] and A. Nishikawa[1]. [1]*Division of Pathology, National Institute of Health Sciences, Tokyo, Japan and* [2]*School of Pharmacy, Iwate Medical University, Iwate, Japan.*

In our short-term study using CAR knockout (KO) mice, it was clarified that piperonyl butoxide (PBO) induces liver hypertrophy without CAR-dependence, while decabromodiphenyl ether (DBDE) induces the lesion in a CAR-dependent manner similarly to phenobarbital (PB). To confirm the involvement of CAR in the

process of hepatocarcinogenesis, 6-week-old male CARKO mice (C3H strain origin) and wild C3H mice were treated with 5,000 ppm PBO, 50,000 ppm DBDE or 500 ppm PB in diet for 27 weeks after the initiation with diethylnitrosamine (DEN). Control animals were fed basal diet alone after DEN initiation. As a result, in wild mice the PBO and PB treatments increased incidences and multiplicities of altered foci and adenomas in the liver, of which phenotypes were mainly eosinophilic. In CARKO mice, PBO and PB drastically reduced such eosinophilic lesions compared with the relevant treated wild mice, and no tumor was induced by PB. In contrast, DBDE mainly induced basophilic lesions in wild mice. Interestingly, multiplicity of basophilic lesions was greater in CARKO mice given DBDE compared with control CARKO mice, and comparable to those in DBDE-treated wild mice. These results indicate that CAR might play an important role in the mechanism of hepatocarcinogenesis induced by PBO or PB, while other mechanisms might exsist as a main pathway of DBDE-induced hepatocarcinogenesis. Taken together with short-term and two-stage carcinogenicity studies using CARKO mice, our results suggest a novel possibility that CAR-mediated hepatocarcinogenesis might follow different process from CAR-mediated liver hypertophy in mice.

PS 2658 DNA DAMAGE MODULATION IN *XPA(-/-)P53(+/-)* AND WILD TYPE C57BL/6 MICE FED BENZO[A]PYRENE WITHOUT OR WITH CHLOROPHYLLIN (CHL) FOR 28 DAYS.

K. John[1], M. I. Churchwell[2], F. A. Beland[2], M. M. Pratt[3], G. McMullen[1] and M. C. Poirier[1]. [1]*Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD,* [2]*Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. FDA, Jefferson, AR and* [3]*National Center for Environmental Assessment, Office of Research and Development, U.S. EPA, Washington, DC.*

Chlorophyllin (CHL), a chemopreventive agent, was previously shown to reduce formation of DNA damage in normal human mammary epithelial cells exposed to BP (John *et al., Cancer Letters*, 2010). Here we examined the effects of CHL on DNA adduct formation in nucleotide excision repair (NER)-deficient [*Xpa(-/-)*] and tumor suppressor haploinsufficient [*p53(+/-)*] mice, and their wild type (WT) counterparts, fed either control diet, or diet containing 100 ppm BP, or 100 ppm BP + 0.3% CHL for 28 days. DNA damage (expressed as adducts per 10^8 nucleotides ± SE, n=6-10 mice/group) was measured in liver, lung and esophagus by chemiluminescence immunoassay (CIA) using antiserum elicited against DNA modified with r7,t8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE). As expected, there was more DNA damage in *Xpa(-/-)p53(+/-)* mice compared to WT mice, confirming the absence of normal NER capacity in the transgenics. In *Xpa(-/-)p53(+/-)* mice fed BP + CHL there was no change in DNA adduct levels in esophagi and lungs, compared to mice fed BP alone. However, in liver the addition of CHL caused a significant reduction [104.9 ± 13.3 for BP vs. 36.7 ± 10.6 for BP + CHL]. In WT mice fed BP + CHL the adduct levels were unchanged in the lungs and significantly increased in esophagi [15.4 ± 1.5 for BP vs. 65.1 ± 8.7 for BP + CHL] and liver [54.5 ± 10.5 for BP vs. 261 ± 20.3 for BP + CHL], compared to mice fed BP alone. Analysis of BPdG adducts by HPLC-MS/MS in *Xpa(-/-)p53(+/-)*and WT mice is currently in progress. The increase in DNA damage in esophagi and livers of WT mice suggest that chronic human use of CHL for chemoprevention of PAH-mediated carcinogenesis should be viewed with caution as the burden of DNA damage may not be lowered in all organs.

PS 2659 EXPOSURE TO URBAN PARTICULATE MATTER INDUCES A DECREASE IN E-CADHERIN EXPRESSION IN MULTICELLULAR TUMOR SPHEROIDS.

F. M. Bernal-Herrera[1], C. M. García-Cuéllar[2], R. Morales-Barcenas[2], Y. Sánchez-Pérez[2] and Y. I. Chirino[1]. [1]*Unidad de Biomedicina, Universidad Nacional Autonoma de Mexico, Edo. Mex, Mexico and* [2]*Subdirección de Investigación Básica, Instituto Nacional de Cancerologia, Mexico, Mexico.* Sponsor: H. Glauert.

Epidemiological studies have demonstrated that exposure to particulate matter (PM) with an aerodynamic diameter smaller than 10 μm (PM10) is associated an augment the risk of lung cancer. To investigate the effects of PM10, several monolayer cell culture models have been studied, however, the PM10 effects on tumor cell models has not been completely explored. The loss of E-cadherin is associated with the invasiveness of tumors, metastatic dissemination and a poor prognosis. We hypothesized that PM10 exposure could induce a decrease in E-cadherin expression in an established tumor. To test this hypothesis, multicellular tumor spheroids (MTS) of lung adenocarcinoma cells were grown in 96-plates (40,000 cell per well in non-adherent conditions) for 4 days. On day 5, spheroids were exposed to PM10 (5 μg/mL, 10 μg/mL and 20 μg/mL) for 24 h. Then, spheroids were collected to western blot analysis. The expression of E-cadherin, actin and GAPDH were performed. We compared the effect of PM10 exposure on the expression of above mentioned proteins in monolayer cell culture of the same cells. Our results show that PM10 induces a 20% decrease in E-cadherin expression in the higher concentration in MTS but monolayer cell culture remains without changes. We also found that PM10 induces a 10% increase in actin expression in MTS (10 μg/mL and 20 μg/mL) and using phalloidin, a dynamic actin polymerization to filamentous actin was observed by confocal microscopy in monolayer cell culture. The expression of GAPDH was used as loading control. Based on these results, we conclude that PM10 induced a decrease in E-cadherin expression in MTS and PM10 also induced an increase in actin expression that could be related to changes to filamentous actin.

PS 2660 STUDIES ON METABOLISM OF 1, 4-DIOXANE.

W. Eck[1], J. Fishbein[2], B. Ginevan[2], N. Koissi[2] and N. Shah[2]. [1]*Directorate of Toxicology, U.S. Army Public Health Command, Aberdeen Proving Ground, MD and* [2]*Department of Chemistry and Biochemistry, University of Maryland—Baltimore County, Linthicum, MD.*

1,4-Dioxane is a water-miscible, semi-volatile organic compound that is found in ground water at many military installations as a consequence of being used as a stabilizer for the degreasing solvent 1,1,1-trichloroethane. Dioxane use continues for production of small arms ammunition and as a component of various other formulations. The U.S. Army is facing a significant environmental restoration liability because of dioxane contamination. The carcinogenic potential of dioxane is dependent upon its mode of action and the role of dioxane metabolites. Two possible metabolites have previously been identified—1,4-dioxan-2-one (dioxanone) and 2-hydroxyethoxyacetic acid (HEAA); the former with electrophilic properties capable of genotoxicity. Questions have remained about the potential role and relationship between the two metabolites, which can exist in a simple equilibrium with one another. To better understand the thermodynamic and kinetic parameters of the equilibrium between dioxanone and HEAA, an analysis of the excreted metabolites without chemical derivatization was conducted. The equilibrium constant for the reaction between dioxanone and HEAA was found to greatly favor HEAA. Kinetic analysis found that the reaction was rapid, with the half-life of the hydrolysis reaction being approximately 0.4 hours. Further in vivo metabolism was studied in Sprague-Dawley rats. Analysis of blood and urine samples for up to 8 hours after oral gavage failed to detect the presence of any dioxanone in either medium, an observation that is consistent with the physico-chemical parameters of the reaction. These data suggest that there is a low probability that the dioxanone electrophillic metabolite is created and responsible for genotoxic induction of cancer.

PS 2661 THE *IN VIVO* ROLE OF ADIPOCYTE-SPECIFIC PPARγ IN ENVIRONMENTAL RISK FACTOR-MEDIATED BREAST TUMOURIGENESIS.

G. Skelhorne-Gross[1], N. Peterson[1], R. Rubino[1], S. SenGupta[1] and C. Nicol[1, 2]. [1]*Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada and* [2]*Division of Cancer Biology and Genetics, Queen's University, Kingston, ON, Canada.*

One in 28 women will die from the growth and spread of breast cancer. The interactions between genetic and environmental (chemical and western-style high fat diet (HF)) risk factors in this process remain unclear. HF consumption also correlates with increased secretion of adipocyte signaling factors linked to breast tumourigenesis. Peroxisome proliferator-activated receptor (PPAR)γ plays a role in many cancers and controls the expression of genes essential for normal fat and sugar metabolism. We have shown using PPARγ heterozygous (+/-) mice that PPARγ normally stops the *in vivo* progression of 7,12-dimethylbenz[a]anthracene (DMBA)-mediated breast tumours. Since many breast cell types express PPARγ, each with unique signal patterns, we aim to define which are required for these PPARγ-dependent effects. Here we examine the hypothesis that adipocyte-specific PPARγ signaling stops the progression of multi-risk factor (DMBA+HF)-mediated breast tumourigenesis. To do this, we generated PPARγ(+/-) and adipocyte-specific PPARγ knockout (PPARγ-A KO) mice and their wild-type (WT) controls. Eight week old female mice (n=10, n=12, n=25, respectively) were treated p.o. with 1mg DMBA once/week and given HF for the duration of the study (25 weeks). Our data show that DMBA+HF treated PPARγ-WT mice have significantly decreased overall survival and tumour latency (13 vs 25 weeks), and increased mammary (43% vs 18%) and skin (86% vs 64%) tumour multiplicity compared to DMBA+normal diet treated controls ($p<0.05$). PPARγ(+/-) and PPARγ-A KO studies are still in progress. Further, serum leptin levels are significantly increased

($p<0.05$) among untreated PPARγ(+/-) and PPARγ-A KO mice compared to PPARγ-WTs. Our *in vivo* data are the first to link normal PPARγ signaling in adipocytes and regulation of pro-tumourigenic leptin levels as a mechanism to stop environmental risk factor-mediated breast tumourigenesis.

S 2662 DEVELOPMENTAL EXPOSURE TO ENVIRONMENTAL TOXICANTS: FROM PERSISTENT TOXICITIES TO DISEASES.

M. La Merrill[1], T. Simones[2], B. Hannas[3], J. Larocca[4], S. Li[5], B. Winans[6], K. M. Streifel[7], E. Tokar[8] and E. Kendig[9]. *[1]Mount Sinai School of Medicine, New York, NY, [2]University of Montana, Missoula, MT, [3]U.S. EPA, Research Triangle Park, NC, [4]Brown University, Providence, RI, [5]University of Georgia, Athens, GA, [6]University of Rochester, Rochester, NY, [7]Colorado State University, Fort Collins, Co., [8]National Toxicology Program, Research Triangle Park, NC and [9]University of Cincinnati, Cincinnati, OH.*

Ontogenesis involves extensive stem cell expansion and differentiation to create the physical architecture of tissues. This process is mediated by the coordination of multiple developmental pathways that involve a large number of key molecular regulators. Deregulation of these pathways may lead to chronic diseases, such as obesity, diabetes, cancer, and cardiovascular disease, which altogether are responsible for the majority of deaths in the United States. Recent experimental evidence suggest that if a developing organism is subjected to certain stressors, including exposure to various environmental toxicants, during the highly susceptible window of ontogenesis, the physical architecture and molecular milieu may be persistently altered, leading to dysfunction and disease in the adults. Correlations have been established between developmental exposure to certain environmental toxicants and adult diseases. Novel molecular and cellular approaches are being utilized to elucidate the mechanisms by which environmental toxicants disrupt developmental pathways and cause adult diseases. We will demonstrate both the breadth and the depth of the most cutting edge research in the toxicities and molecular mechanisms that support this emerging paradigm. Persistent and novel toxicities resulting from developmental exposures to environmental toxicants of human relevance, including halogenated hydrocarbons, metals, and phenols, will be presented in this symposium. In summary, our attention will be devoted to focusing on how these exposures during development give rise to long-term adverse effects or disease outcomes in endocrine, immune, nervous, metabolic, and reproductive systems.

S 2663 INVESTIGATION OF THE MECHANISM FOR PHTHALATE-INDUCED TOXICITY DURING MALE SEXUAL DIFFERENTIATION IN THE RAT.

B. Hannas, J. Furr, C. Lambright, V. S. Wilson and L. Gray. *Reproductive Toxicology Branch, U.S. EPA, Research Triangle Park, NC.*

Male rats exposed to phthalate esters during sexual differentiation (GD14-GD18) display various reproductive developmental abnormalities later in adult life which are associated with declines in fetal testicular testosterone (T) production and insulin-like three hormone (Insl-3) gene expression levels. Little is known about the precise mechanism behind phthalate-induced testosterone and Insl-3 gene expression declines. The goal of this study was to identify the proximate mechanism of toxicity induced by in utero phthalate exposure by evaluating candidate signaling pathways. Our approach was to identify the earliest time point at which we could detect a significant decline in fetal testicular T production following a single maternal dose of dipentyl phthalate (DPP). We determined that GD 16 is the earliest day that we could detect fetal testicular T production by radioimmunoassay. Sex differences in whole body T levels could be detected one day earlier (GD15). Next we performed dose response and time course studies and demonstrated that an oral dose of 850 mg/kg DPP results in significant T production decline on GD17 and 3 hours following maternal dosing. We will continue to evaluate this time point by assessing the dose-response of T production to DPP following dosing. Ongoing studies will interrogate toxicity pathways by analyzing response expression of target genes using RT-PCR arrays. Additionally, we assessed early postnatal effects of in utero DPP exposure after dosing dams with 0, 11, 33, 100, or 300 mg/kg/d DPP throughout GD8-18. Evaluation at postnatal day (PND) 2 revealed a clear dose-responsive decline in male anogenital distance (AGD) and increase in nipple retention incidence on PND 13. Together, these studies will facilitate additional investigations for elucidating the protein or gene-level mechanism behind the reproductive toxicity associated with phthalates and enable us to correlate fetal hormone and gene alterations with the postnatal effects. Disclaimer: This abstract does not necessarily reflect EPA policy.

S 2664 ROLE OF NEUROINFLAMMATION IN DEVELOPMENTAL VULNERABILITY TO MANGANESE.

K. Streifel, J. Moreno and R. Tjalkens. *Toxicology Program, Center for Environmental Medicine, Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO.*

Chronic environmental exposure to manganese (Mn) can lead to a degenerative neurological disorder termed manganism. There have been a multitude of adult exposure studies, however much less is know about exposures to the developing CNS. We previously reported that the developing CNS is highly sensitive to Mn-induced neuroinflammatory changes in glial cells, resulting in increased expression of inducible Nitric Oxide Synthase (NOS2) in astrocytes and microglia, with a concomitant increase in neuronal protein nitration throughout the basal ganglia (Moreno et al., 2009). Based upon these data, we postulated that juvenile mice lacking NOS2 would be protected against the neurotoxic effects of Mn and exhibit decreased gliosis and nitrosative stress in neurons. We therefore exposed NOS2 knockout mice or wildtype controls to determine if loss of this gene protects against Mn neurotoxicity in vivo and in vitro. Mice were administered 50 mg/kg MnCl2 daily by intragastric gavage from day 21- 34 postnatal and evaluated for locomotor function, catecholamine levels, glial activation, neuronal protein nitration and neuropathology. These data demonstrate that NOS2 is causally involved in Mn-induced neurotoxicity in developing mice and suggests that neuronal protein nitration is an important determinant of neuronal injury from Mn. These data also suggest that persistent neuroinflammation from developmental Mn exposure could have long-term impacts on neuronal function.

S 2665 THE ROLE OF STEM CELLS IN ARSENIC-INDUCED TRANSPLACENTAL CARCINOGENESIS.

E. J. Tokar and M. P. Waalkes. *NTP, NIEHS, NIH, Research Triangle Park, NC.*

Emerging evidence indicates that cancer stem cells (CSCs) are the true malignant cells within tumors and are carcinogenically initiated from normal stem cells. We find that inorganic arsenic exposure *in utero* causes cancer in mice much later in life as adults. This stunningly delayed response suggests that a stem cell population is targeted by arsenic, possibly producing quiescent CSCs that then produce cancers in adult life. Transplacental carcinogenic activity may also occur with arsenic in humans. In mice, we find that fetal arsenic exposure causes or initiates tumors at various sites and the tumors very often show a remarkable CSC overabundance compared to tumors induced by organic agents or spontaneous tumors. This CSC overabundance appears to correlate with an arsenic-induced survival selection for target tissue-relevant stem cells that we have observed with stem cell lines *in vitro*. During this survival selection arsenic-induced malignant transformation of human and rodent stem cells occurs. Primary events and genetic alterations involved in this arsenic initiation of CSCs include early loss (< 9 weeks) of stem cell self-renewal gene expression (*p63, ABCG2, BMI-1, SHH, OCT4, NOTCH-1*) but then re-expression as the tumor suppressor gene *PTEN* is progressively suppressed (> 9 weeks) and CSC phenotype acquired. This phenotype appears to be associated with aberrant expression of at least some imprinted genes. This indicates that arsenic, a ubiquitous environmental contaminant and known human carcinogen, directly transforms stem cells into pluripotent CSCs. Thus, stem cells appear to be a primary target for arsenic-induced transplacental carcinogenic initiation.

S 2666 ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR REPROGRAMS THE DEVELOPING IMMUNE SYSTEM.

B. Winans, S. R. Marr and B. Lawrence. *Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY.*

It is becoming increasingly recognized that early life exposure to environmental agents can lead to persistent alterations in immune function, but the mechanism by which this occurs is not yet known. To investigate the susceptibility of the developing immune system to reprogramming, our laboratory uses 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a model developmental immunotoxicant and influenza A virus as a prototypical human pathogen. TCDD is a ubiquitous pollutant, and represents a category of agents that activate the aryl hydrocarbon receptor (AhR), which recent studies suggest plays a role in developmental regulation. Our laboratory has demonstrated that developmental activation of AhR leads to persistent alterations in the immune response to influenza A virus in adult mice; specifically, there is a reduced clonal expansion and differentiation of $CD8^+$ T cells. Further, this alteration results from a direct effect on hematopoietic cells. These findings show that sustained AhR activation programs the developing immune system, leading to persistent functional deregulation. Given that this change in

CD8+ T cell function occurs without a detectable change in lymphoid organ cellularity or in the distribution of immune cell subpopulations, we hypothesize that inappropriate AhR activation during development alters epigenetic regulatory mechanisms such that the response of T lymphocytes is permanently altered. This represents a new paradigm in developmental immunotoxicology, suggesting that AhR influences epigenetic control of immune function.

S 2667 THE EFFECTS OF ORAL BISPHENOL A EXPOSURE IN THE DEVELOPING CD1 AND C57BL/6 MOUSE.

E. Kendig, S. Christie, C. Cookman, C. Lo, D. Buesing, T. McCutchan, J. Cooper and S. Belcher. *Pharmacology and Cell Biophysics, University of Cincinnati, Cincinnati, OH.*

The endocrine disrupting effects of Bisphenol A (BPA) have been demonstrated in various mammalian and non-mammalian model systems. However, there is some inconsistency in the ability to replicate results. The significance of studies demonstrating harmful effects of BPA and those reporting little or no effect have been highly scrutinized and become the subject of much controversy. Here we report findings from preliminary studies designed to assess the comparative effects of pre- and postnatal dietary exposure of BPA and 17α-ethinyl estradiol on various developmental, reproductive and cardiovascular endpoints in developing and adult C57Bl/6 and, CD1 (swiss) mice. Preliminary results indicate clear sex- and strain-specific differences in sensitivity to treatment-related changes in measures of fertility. These data also suggest potential cardiovascular effects of treatment with BPA including altered heart rate and the promotion of arrhythmogenesis, suggestive of altered calcium dynamics in the heart. The sensitivity of the mouse strain used appears to be a significant variable in the study of developmental toxicity of endocrine disruptors, suggesting that strain selection is a significant variable for each endpoint of interest.

S 2668 A BBDR-HPT AXIS MODEL FOR THE LACTATING RAT AND NURSING PUP: EVALUATION OF IODIDE DEFICIENCY.

S. Li[1] and J. W. Fisher[2]. [1]*Biostatistics, Medical College of Georgia, Augusta, GA and* [2]*Food & Drug Administration, Jefferson, AR.*

The hypothalamic-pituitary-thyroid (HPT) axis controls many physiologic functions, including metabolism, growth, development and reproduction. However, there is no adequate risk assessment tools exist to evaluate the health outcomes of chemical/dietary induced changed changes in serum thyroxine (T4, T3) concentrations in either euthyroid or sensitive populations. Ongoing collaborative studies are underway examining the effects of iodide deficiency on CNS development in the rat pup at the USEPA (Drs Mary Gilbert and Kevin Crofton). Electrophysiological, biochemical and molecular studies of the pup brain combined with behavioral studies are used for evaluating HPT mediated developmental neurotoxicity. Our Biologically Based Dose Response (BBDR) HPT axis model for the lactating rat and nursing pups aims to better understand and predict the relationship between brain concentration of T3 and serum concentration of T4. We also investigated the effects of two negative feedback loops: the first being TSH production and brain concentration of T3, and the second being serum TSH stimulation of the thyroid sodium iodide symporter (NIS) and thyroid hormone (T3 and T4) synthesis and production under both sufficient iodine and deficiency iodine uptake conditions. The model is calibrated to predict dietary iodine deficiency-induced perturbation in serum and brain thyroid hormones (regulation of deiodinase I, II and III enzymes) during the nursing period. After successful building of the BBDR model for iodide deficiency, the model will be expanded to include thyroid active chemicals. For example, we have conducted several studies with perchlorate and PCB126, both are thyroid active chemicals, with fairly well defined modes of action on the HPT axis. These models will allow for simulation of complex dose response and exposure conditions that are likely to result in adverse developmental neurotoxicty and ultimately serve as a template for the future development of human gestation and lactation BBDR HPT axis models for use in risk assessment.

S 2669 VASCULAR INJURY: A FIGMENT OF YOUR INFLAMMATION?

H. W. Smith[1] and M. P. Lawton[2]. [1]*Eli Lilly and Company, Indianapolis, IN and* [2]*Pfizer, Groton, CT.*

Because drug-induced vascular injury (DIVI) is a process rather than a single disease entity, diagnosing and predicting it is like chasing an apparition—there are many sightings but the descriptions are often different. The preclinical finding of vascular injury was initially described in rats and dogs, but has also been observed in mice and non-human primates in recent years. While vasculitis in humans appears to be immune-related, the triggers that induce blood vessels to become damaged and inflamed in animals have not been determined. In addition, it is difficult to distinguish the primary event from secondary inflammation that accompanies the induced damage. In animals, the injury can be observed within hours of drug exposure, whereas in humans it may not appear for weeks or months. With the disparity of observations between preclinical species and humans, it is unclear whether drugs that cause vascular injury or immune-mediated vasculitis in animals do so in humans, and whether a finding of preclinical DIVI predicts the risk of vascular injury in humans. As pharmaceutical companies expand their drug discovery efforts into new chemical spaces, higher incidences of preclinical DIVI are being observed in a broader variety of organs, which continue to impede drug development. If DIVI in animals represents a real hazard to humans, it is paramount for the pharmaceutical industry to develop means for early detection and monitoring of vascular injury. An overview of the current understanding of the pathobiology of vascular injury will be provided and the challenges in diagnosing and predicting DIVI with new advances in biomarker strategies will be discussed. In an exploration of the impact of DIVI on drug development, investigative models and case studies will be shared using examples of small and large molecule-induced vascular injury in animals.

S 2670 THE PATHOBIOLOGY OF VASCULAR INJURY.

J. R. Turk. *Amgen, Thousand Oaks, CA.* Sponsor: M. Lawton.

Drugs and xenobiotics may modulate the function of myriad enzymes that impact vasomotor tone, hemostasis, vascular permeability, inflammation, angiogenesis, tissue oxygenation, and metabolic stress. The microscopic anatomy of the vasculature is relatively simple, consisting of three tunicae: (1) intima, composed of endothelial cells; (2) media, composed of smooth muscle cells and bounded by porous internal and external elastic laminae; and (3) adventitia, composed primarily of fibroblasts, that in epicardial conduit and other larger vessels, are surrounded by and interdigitate with vasa vasorum, and adipose tissue that contains not only adipocytes, but also capillaries, lymphocytes, macrophages, mast cell/basophils, neutrophils, and nerves. Each of these cell types may express potential biomarkers of injury associated with characteristic histopathologic lesions. Biomarkers are sought for those that may precede and predict characteristic changes on histopathology. A review of extant knowledge of drug induced vascular injury will be presented.

S 2671 THE IMPACT OF VASCULAR INJURY ON DRUG DEVELOPMENT.

W. Kerns. *Accellient Partners LLC, Harvard, MA.*

Over the past 30 years, countless compounds in development have been labeled as causing vascular injury in healthy animals during the routine conduct of safety studies. Although some of these compounds have been approved as products, a majority have been shelved while industry and academic scientists attempt to define methods to prove that these hazards are not relevant to humans. In humans, there is a background of vascular pathology in most western cultures, making this even more challenging. Proving a negative is a daunting task. The intent of this presentation is to review this drug development problem across the industry and to assess its impact from a cost and lost opportunity perspective.

S 2672 PROGRESS IN IDENTIFYING BIOMARKERS OF VASCULAR INJURY.

B. E. Enerson. *Drug Safety Research and Development, Pfizer, Groton, CT.* Sponsor: M. Lawton.

There is a need for sensitive and specific biomarkers of vascular injury that can be qualified for use in preclinical and clinical settings. In addition, the use of biomarkers of vascular injury in early toxicity studies during drug development has the potential to reduce compound attrition from this difficult to manage finding. Although it is likely there are common pathological processes that contribute to drug-induced vascular injury, this toxicity is complex, with different primary mechanisms of injury that depends on the pharmacological class, off-target profile and/or physical structure of the drug. Therefore selection of biomarkers should be based on identifying a limited but still broad selection of potential markers that cover different mechanisms of injury in different species that are specific, sensitive, and prognostic for vascular injury. This presentation will review recent advances in vascular injury biomarker research and will highlight collaborative efforts towards regulatory acceptance.

S 2673 IMMUNE-MEDIATED VASCULAR INJURY IN NON-HUMAN PRIMATES.

J. L. Bussiere[1] and C. Johnson[2]. [1]*Toxicology, Amgen, Inc., Thousand Oaks, CA and* [2]*Pathology, Amgen, Inc., Thousand Oaks, CA.*

Anti-drug antibody (ADA) responses can potentially affect the outcome and interpretation of a toxicology study and assessing the relevance of toxicity that may be ADA-mediated in nonhuman primates to clinical populations can be difficult. During the development of a monoclonal antibody (MAb) drug which targets the immune system, a vascular lesion was noted that was not immediately explainable as drug or ADA-related. In a 6-week cynomolgus monkey study with weekly dosing of 10, 75 or 300 mpk IV or 300 mpk SC, chronic periarteritis involving multiple organs was observed in 2/6 animals given 300 mpk IV, and in 1 tissue in 1/6 animals given 300 mpk IV, 1/6 animals given 75 mpk IV and in 1/6 animals given 300 mpk SC. The etiology of the vascular lesion was uncertain based on routine histopathology evaluation. A vascular target was not anticipated based on literature describing target-deficient humans or KO mice and was thus thought not to be related to the mechanism of action of the MAb. Evidence of an immune response to the MAb associated with enhanced clearance was noted in 3/5 animals with the lesion, and these animals tended to develop ADA quicker than non-affected animals that eventually became antibody positive. The safety margin at the NOAEL of 10 mpk was ~5x/20x based on AUC/Cmax, and FIH trials were initiated. In a follow up 3-month study with weekly SC dosing of 5, 25, or 90 mpk, periarteritis was noted in only 1 tissue of 1 animal at the mid dose. Immunohistochemical (IHC) staining for antihuman IgG and antimonkey IgG and IgM revealed granular staining in blood vessels with the vascular lesions of animals from both studies. This staining pattern suggested that ADA/drug complex deposits might have a role in the pathogenesis of the lesion. No periarteritis was seen in a 6 month study at similar doses. These data supported the conclusion that ADA contributed to the toxicity seen with this MAb and was not likely to be relevant to humans. Close monitoring of ADA in the clinical trials, along with other endpoints allowed for progression of single and multiple dosing.

S 2674 IMAGING AS A TRANSLATIONAL TOOL FOR ASSESSING CLINICAL RELEVANCE OF DRUG-INDUCED VASCULAR INJURY.

A. Lerman. *Mayo Clinic, Rochester, MN.* Sponsor: H. Smith.

There is a growing need to identify the potential cardiovascular effect of new therapeutic options prior to launching large randomized clinical trials, and particularly introducing the new therapy to clinical practice. The endothelium is strategically located between the circulation and the vascular wall and regulates vascular tone, growth and thrombosis. The vascular wall continues to undergo continuous vascular injury and repair, and the role of the endothelium is to mediate this repair process. An abnormality that impairs this process results in endothelial dysfunction and progresses to more severe vascular injury that can mediate a cardiovascular event and mortality. There are several methodologies to assess the effect of a specific drug on the endothelial function. In vitro methods such as organ baths, to assess the effect of a drug on the vascular function and specifically on the endothelium. Further studies can be done in small and large animals to assess the effect of acutely or chronically administered drug on the vascular endothelial function,. When the studies with the new drug continue into a human investigation, there are several methodologies that enable us to assess the specific effects of this new therapeutic modality on endothelial function in humans. These methods include noninvasive and methods to assess the specific effect on the coronary endothelial function. Additionally, there are novel imaging modality to assess not only the effect on the function, on the structure of the vascular wall. This imaging modality, particularly of interest in the coronary circulation, can have a more accurate imaging of the effect of the drug on the vascular wall. Thus, the application of these in vitro, nonclinical, and clinical methodologies to the assessment of new therapeutic drugs on vascular function and specifically on endothelial function may increase the safety of the drug and prevent the clinical incidence of drug-induced cardiovascular morbidity and mortality.

W 2675 ARE WE THERE YET? – ATTRITION IN THE PHARMACEUTICAL INDUSTRY AND IMPACTFUL STRATEGIES FOR REDUCING FAILURE.

J. Stevens[6], C. Afshari[5], B. Car[1], I. Cotgreave[2], K. Kolaja[3] and B. Pennie[4]. [1]*Bristol-Myers Squibb, Lawrenceville, NJ,* [2]*Astra-Zeneca, Uppsala, Sweden,* [3]*Roche, Nutley, NJ,* [4]*Pfizer, Groton, CT,* [5]*Amgen Inc., Thousand Oaks, CA and* [6]*Lilly, Indianapolis, IN.*

There have been many reports over the years that indicate a significant amount of attrition of candidate molecules in the pharmaceutical industry is due to safety related failures. In the recent years new approaches have been deployed to shift safety related attrition earlier in the pipeline. The impact of these new strategies will not be fully realized until they are in place for a number of years. In the meantime, there is no way to objectively assess to what extent and in what manner we are improving as an industry and what further changes and areas of research are needed. Therefore it is important to begin dialogue that aims to provide a forum to discuss contemporary views of the rate and causes of current safety related pharmaceutical attrition. An analysis of the strategies that appear to be most useful (or least useful) to drive this attrition earlier, i.e. fail fast will be included. Varying perspectives will be provided on the primary reasons for safety related attrition within companies and the current strategies deployed to address the problem. Special focus will be given to the early stage portfolio work through the first in human trials. The strategies that will be discussed will include the integration of in vitro and in vivo models with technologies such as imaging platforms, genomics, and protein/biochemical analyses. Gaps or areas for future research support will be highlighted as appropriate.

W 2676 THE INTEGRATION OF MULTI-DISCIPLINARY APPROACHES WITH DISCOVERY TOXICOLOGY IN OPTIMIZING DRUG CANDIDATES.

B. D. Car. *Pharmaceutical Candidate Optimization, Bristol-Myers Squibb, Princton, NJ.*

Discovery Toxicology has been integral to the drug discovery and development process at BMS and legacy companies for over 13 years. This department approaches target and candidate selection with a multi-disciplinary team including pathologists, cardiologists/safety pharmacologists, toxicologists, molecular and cell biologists. DT has leveraged the integration of classic approaches such as in vivo toxicology and pathology with new technologies including genomics and metabonomics approaches and close partnerships with Discovery Metabolism and Pharmacokinetics, Biotransformation, Bioanalytical and Pharmaceutics groups. Focusing initially on in silico and in vitro technologies, the group moved to a more integrated in vitro/vivo approach to addressing failures due to preventable developability issues and avoidable target-associated safety issues. Collectively, this improved target success and resulted in a lower compound attrition rate. Recognizing that such an approach can create a checkbox mentality and reduce hypothesis testing, the focus has shifted to more a risk-adjusted and therefore fiscally responsible direction, which was achieved without compromising attrition. Key to the success has been high level integration across associated disciplines and within the context of expert chemistry and pharmacology groups, leveraging their expertise whenever possible.

W 2677 POTENTIAL USES OF STEM CELL TECHNOLOGY IN EARLY PIPELINE SAFETY SCREENING.

I. Cotgreave. *Global Safety Assessment, Molecular Toxicology, AstraZeneca, Södertälje, Sweden.* Sponsor: C. Afshari.

Strategies to reduce late- stage attrition are associated with a need to develop and explore novel systems for lead selection. One area that is under exploration is the optimal use for stem cell models. Current attrition reasons and strategies to reduce at Astra-Zeneca will be discussed. Strategies to produce safety screening techniques based on human embryonic stem cells and induced pluripotency will be discussed, focusing primarily on the need to predict human cadio- and hepatotoxicity. Several collaborative initiatives will be high-lighted, including the Stem Cells for Safety of Medicine (SC4SM) program, a UK-based public-private consortium. Results of initial validation exercises will discussed and the expectations for the near future debated.

W 2678 EFFECTIVE AND EMERGING PLATFORMS FOR PREDICTIVE TOXICOLOGY.

K. L. Kolaja. *Nonclinical Safety, F. Hoffmann-LaRoche, Nutley, NJ.*

Attrition of drug candidates within the pharmaceutical industry occurs throughout the entire development process. Ideal screening strategies identify liabilities early and accurately to enable project teams to make lead optimization decisions that will match the clinical goals for a given program. A combination of effective assays that survey genetic toxicology, teratogenicity, cardioavascular toxicity, and target organ

toxicity are blended on a per project team basis to glean the insight of a compounds pharmacological and toxicology profile. Current attrition events in early discovery and the strategies to reduce them at Roche will be discussed.

W 2679 MULTI-PARAMETRIC APPROACHES TO THE PROBABILISTIC ASSESSMENT OF TOXICITY ACROSS CHEMICAL SERIES IN DRUG DISCOVERY.

W. D. Pennie. *Compound Safety Prediction, Pfizer Inc., Groton, CT.* Sponsor: C. Afshari.

Safety-related attrition continues to be a challenging problem in drug discovery and development, accounting for a significant percentage of failures, particularly in the discovery and pre-clinical development stages. In vivo assessment of potential toxicity of early hits is neither practical nor financially possible given the rapidly changing chemistry space in the design stages. To help address this gap we have developed a multi-parameter panel approach to characterizing potential tox risk. The model we have developed consists of both "virtual" (in silico prediction data, physical chemical space parameters, structure toxicity rules) and "real" experimental data. The experimental data is derived from multiple in vitro assessments including genotoxicity, cytotoxicity, safety pharmacology endpoints, secondary pharmacology assays for promiscuity and specific mechanistic assays such as mitochondrial dysfunction, apoptosis etc . We have trained the predictive algorithms against approximately 500 pharmaceutically-active compounds, which have gone through acute rodent toxicology studies. Using this approach we have developed a platform that allows us to correctly identify around two thirds of compounds which will cause toxicity findings at relatively low dose levels (10uM total exposure). The model performs with an acceptable false negative rate of less than 10% and is positioned to allow medicinal chemists to get an early readout of potential tox, helping to differentiate early chemical series, and introducing safety as a viable design parameter for the medicinal chemist.

W 2680 SAFETY RELATED DELAYS AND ATTRITION RATES GUIDE PREDICTIVE AND DISCOVERY TOXICOLOGY STRATEGIES.

C. Afshari. *Comparative Biology and Safety Sciences, Discovery Toxicology, Amgen Inc., Thousand Oaks, CA.*

Typically attrition in the pharmaceutical industry is believed to be affiliated mostly with small molecule candidates. However, we are learning there also is attrition that is associated with large molecule candidates. Analysis of reasons for compound attrition but also safety related issues that occur within our portfolio has served as the basis for our strategy and prioritization of Discovery and Predictive Toxicology assay development and deployment. A key part of the strategy has been to fully characterize the biological activity associated with target modulation. Another important part of this strategy has involved a flexible approach that allows the customized use of various assays within individual projects at various stages of screening and lead optimization. A necessity of this approach has been the need to highly integrate partnership of molecular toxicologists and pathology within these early project teams. Lastly our strategy requires regular collection of data related to compound delay and failure and assessment of impact of current strategies and/or the need to modify. This talk will review our recent assessment and response to our attrition analysis for projects through first in human studies for both large and small molecules. Projection on the general utility of this approach or application to sectors outside the pharmaceutical industry will be proposed.

W 2681 INTEGRATION OF *IN VITRO* AND *IN SILICO* APPROACHES INTO AN ATTRITION-DRIVEN EARLY SAFETY ASSESSMENT STRATEGY.

J. L. Stevens. *Toxicology, Lilly Research Laboratory, Indianapolis, IN.*

Predictive toxicology tools, such as in vitro and in silico models, can be integrated into effective early safety screening for drug-like chemical scaffolds. In our experience, attrition-driven strategies are more useful than global safety screens, i.e. target the approaches to those issues most likely to drive terminations. Success requires consolidation of attrition data from prior efforts with surrogate and molecular endpoints to sharpen the focus. Example where the use of in silico, cell-based surrogate, biomarker and molecular data have been integrated to select improved Lead scaffolds will be discussed.

W 2682 AUTOIMMUNITY *VERSUS* SYSTEMIC HYPERSENSITIVITY: COMMONALITIES USEFUL FOR IMMUNOTOXICITY TESTING.

R. Pieters[1] and D. Germolec[2]. [1]*IRAS, Utrecht University, Utrecht, Netherlands and* [2]*Toxicology Branch, National Toxicology Program, NIEHS, Research Triangle Park, NC.*

Chemical-induced systemic hypersensitivity and autoimmunity are immunotoxicological phenomena that concern chemicals ranging from drugs to environmental pollutants. These phenomena are difficult to predict, in part due to lack of knowledge of underlying mechanisms, although neoantigen-specific immune responses co-stimulated by signals from the innate immune system combined with complex genetic susceptibilities are considered crucial. In case of systemic hypersensitivity to drugs, i.e. drug allergy, immune responses to non-self drug-modified antigens may be intermingled with responses to self-antigens. Immune responses against self-antigens are hallmark of autoimmune diseases; however, a low level of self-reactivity is present in healthy individuals and is considered important for immunological homeostasis. The distinction between allergic and autoimmune responses induced by chemicals may be a very fine line, reflecting the relative antigenicity of the novel- and self-proteins involved. Importantly, development of allergic or autoimmune clinical phenomena, including nature and type, may depend on individual susceptibilities (i.e. genetic polymorphisms), or coinciding environmental triggers (e.g. microbial interactions). The complex etiology and lack of mechanistic knowledge is a challenge for risk assessment as at present no single model is available that may predict chemical-induced autoimmunity and systemic allergy. Hazard and risk assessment of these phenomena will likely require a more holistic translational approach. Exploring the similarities and differences between chemical-induced autoimmunity and systemic allergy is an important step to establish a methodological framework to identify chemicals with the potential to induce these immune system toxicities. Therefore we will focus on the complex relationship between chemical-induced autoimmunity and systemic allergy and the identification of methods, mechanistic commonalities and strategies that would be useful for risk assessment.

W 2683 AUTOIMMUNITY AND AUTOIMMUNE DISEASE.

N. Rose. *Johns Hopkins University, Baltimore, MD.* Sponsor: R. Pieters.

In an immune system designed to recognize the universe of antigenic determinants, it is inevitable that antigens of the host will also be recognized. Thus, autoimmunity in the form of self-reactive T and B cells arises and is demonstrable in all normal individuals. These autoimmune responses are kept under control by a collection of contrivances. When these regulatory mechanisms fail, benign autoimmunity can progress to pathogenic autoimmune disease. The process is cumulative with check points along the way. We have analyzed the role of environmental agents on three different models of autoimmune disease in mice: thyroiditis, myocarditis and hepatitis. In all three models, environmental effects are determined by the genetics of the host and the context in which the environmental agent is presented. The initial encounter with the innate immune system dictates the later course of events on the basis of the cell populations stimulated and the mediators produced. The subsequent inflammatory response, a consequence of adaptive CD4+ T cell dependent immunity, is reflected in the profile of cytokines/chemokines produced. In severe instances of autoimmune disease, the inflammatory process transitions to irreversible fibrosis. Knowledge of the critical check points offers opportunities for earlier detection and remediable intervention to arrest pathogenic autoimmunity.

W 2684 AUTOIMMUNITY *VS.* ALLERGY: A CRITICAL NEED IN SAFETY TESTING.

R. R. Dietert. *Microbiology and Immunology, Cornell University, Ithaca, NY.*

The prevalence of childhood allergic and autoimmune diseases has increased dramatically in recent decades placing immune-based diseases among the foremost pediatric concerns. Required medical care, including hospital visits, and therapeutic drug use have increased in kind with the rise in these diseases. Yet safety testing to identify the environmental hazards for these diseases has not adapted to adequately address this emerging health risk. For this reason, there is an urgent need to expand the scope of routine immunotoxicity testing well beyond the historic focus on immunosuppression and identification of classic sensitizing chemicals. But assessment of allergy- and autoimmune-associated immune dysfunction can be challenging in immunotoxicity testing. Gene-environment interactions play a significant role in these diseases and contribute to this assessment challenge. Not all genotypes are equally susceptible to drug- and chemical-induced immune dysfunction associated with allergy and autoimmunity. As a result, test systems offer different capabilities

for risk identification and characterization relative to allergic and autoimmune disease (e.g., examining general vs. more susceptible subpopulations and disease induction vs. disease exacerbation). This presentation will discuss allergic vs. autoimmune responses, potential biomarkers for identifying chemical and drug hazards, the importance of inflammation in these responses and options for revised safety testing that could improve risk assessment for these chronic conditions.

W 2685 MECHANISMS UNDERLYING HEXACHLOROBENZENE-INDUCED IMMUNOTOXICITY: COMPARISON WITH IDIOSYNCRATIC DRUG REACTIONS.

J. Ezendam[1] and R. Pieters[2]. [1]*National Institute for Public Health and the Environment, Bilthoven, Netherlands and* [2]*Institute for Risk Assessment Sciences, Utrecht, Netherlands.*

The toxicity of hexachlorobenzene (HCB) was discovered in the 1950s after an accidental poisoning in Turkey. Some symptoms, such as enlarged lymph nodes and arthritis, were indicative for immunotoxicity. In rats, orally exposed to HCB several immunological parameters were enhanced, including increased spleen and lymph node weights, enhancement of humoral responses and induction of inflammatory skin and lung lesions. The increased autoantibody levels and modulation of experimental autoimmune diseases might indicate that HCB could induce autoimmunity, similar to idiosyncratic drug reactions. Drugs like D-penicillamine and nevirapine induce skin lesions and autoimmune phenomena as well. In the etiology of these diseases T cells and macrophages are involved. The role of macrophages and T cells in HCB-induced immunostimulation has been studied in Brown Norways (BN) rats. After depletion of T cells with Cyclosporin A (CsA) in rats orally exposed to HCB, the onset of skin lesion was delayed and splenomegaly was prevented partly. The rise in IgE and autoantibody levels and lung eosinophilia were prevented completely. However, adoptive transfer experiments showed that the disease could not be transferred to naïve recipients, demonstrating that HCB does not induce specific T cell sensitization. Depletion of macrophages with clodronate-liposomes prevented of HCB-induced skin and lung lesions partly and inhibited elevation of autoantibodies. The involvement of the innate immune system in HCB-induced immune effects was further confirmed in a microarray study, demonstrating that HCB activated several innate immune pathways. In conclusion, HCB exposure induced a systemic inflammatory response, probably triggered by macrophage activation. Subsequently, T cells are polyclonally activated and clinical signs develop. In contrast, idiosyncratic reactions involve macrophages and T cells as well, but this disease is induced after specific T cell sensitization.

W 2686 AUTOIMMUNITY IN IDIOSYNCRATIC DRUG REACTIONS.

J. Uetrecht. *University of Toronto, Toronto, ON, Canada.*

Idiosyncratic drug reactions (IDRs) represent a major problem because at present there is no way to accurately predict which drug candidates will cause such reactions nor which patients are at risk. This unpredictability is largely due to a lack of mechanistic understanding. Many IDRs are clearly immune-mediated but this is not always the case, and some have characteristics that are atypical for an immune response. Most drugs associated with a high incidence of IDRs can cause different types of IDRs in different people, e.g. propylthiouracil can cause liver injury, agranulocytosis or a lupus-like autoimmune syndrome. On a molecular level, drugs induce a mixture of antibodies: some against various drug-modified proteins and also autoantibodies against native proteins with a different pattern in each patient. This is not surprising because each person has a different repertoire of major histocompatibility antigens and T cell receptors, and it is expected that the highest affinity recognition would be different in each individual. The typical characteristics of an immune-mediated reaction include a delay between starting the drug and the onset of the adverse event but a rapid onset with rechallenge. The time to onset varies with the type of IDR with rashes typically occurring after a week or so, liver injury after a month or so and autoimmune IDRs often requiring more than a year of drug exposure before the onset of autoimmunity. This long delay in the onset of an autoimmune reaction is presumably due to mechanisms that usually prevent autoimmunity. In addition, autoimmune responses are not always associated with an amnestic response, possibly for the same reason. Two animal models of drug induced autoimmunity: penicillamine in rats and propylthiouracil in cats do not display an amnestic response. Thus the variability in the nature of IDRs is most easily explained by an immune mechanism, and features such as a long delay in onset and a lack of amnestic response are consistent with an autoimmune component. Valid animal models would greatly facilitate mechanistic studies but variability also makes development of such models a challenge. Supported by grants from CIHR.

W 2687 TRICHLOROETHYLENE-INDUCED AUTOIMMUNITY; DEPENDENCE ON METABOLISM AND GENETIC SUSCEPTIBILITY.

K. Gilbert[1, 2]. [1]*Microbiology and Immunology, Arkansas Children's Hospital Research Institute, Little Rock, AR and* [2]*University of Arkansas for Medical Sciences, Little Rock, AR.*

MRL +/+ mice exposed to occupationally relevant doses of the environmental contaminant trichloroethylene (TCE) for 32 weeks developed T cell-mediated autoimmune hepatitis, accompanied by the expansion of IFN-γ-producing and apoptosis-resistant ($CD44^{hi}$ $CD62L^{lo}$) $CD4^+$ T cells. TCE-induced alteration in $CD4^+$ cell activity required metabolism, and could be mimicked by exposure to the TCE metabolite trichloroacetaldehyde hydrate (TCAH). However, TCAH did not induce autoimmune hepatitis, but instead promoted skin inflammation and hair loss similar to autoimmune alopecia in MRL+/+ mice. Unlike MRL+/+ mice, genetically-related C3H/HeJ mice exposed to TCAH did not develop alopecia or altered $CD4^+$ T cell function. Interestingly, microarray analysis revealed that $CD4^+$ T cells from MRL+/+ mice had a much higher constitutive expression of the osteopontin-coding gene Spp1 than cells from C3H/HeJ mice. TCAH appears to further increase expression of Spp1 as well as genes for other proinflammatory cytokines (e.g. IFN-γ). Thus, it appears that altered constitutive as well as toxicant-induced expression of certain $CD4^+$ T cell genes may increase susceptibility to the immunotoxicity of TCE and its metabolite TCAH.

W 2688 PBPK MODEL USE IN RISK ASSESSMENT: WHY BEING PUBLISHED IS NOT ENOUGH.

E. D. McLanahan. *ORD/NCEA, U.S. EPA, Research Triangle Park, NC.*

While publication of a physiologically-based pharmacokinetic (PBPK) model in a peer-review journal is a mark of good science, evaluation of published models and the supporting computer code can identify issues or shortcomings that can be barriers to their use in risk assessment. The quality of human health risk assessments must be sustained, including use of appropriate PBPK models and other quantitative tools for dose-response evaluation and interspecies extrapolation. A published model can be modified to address assessment-specific issues, and having clear criteria can reduce the number of review and revision iterations and hence the time needed to bring a model to application. As probabilistic (population) PBPK models are being implemented, parameter choices to fully characterize population variability become critical. Thus a thorough but efficient process for the review and implementation of PBPK models is necessary. While a typical process and set of criteria were created specifically for PBPK models, the principles may be applied to other types of computational models (e.g., dose-response) as they are developed. We have convened a panel of experts in biologically-based modeling and relevant quantitative methods to not only describe and discuss model evaluation and criteria, but also issues and choices that arise in model application and assuring reasonable certainty, as well as computational tools now available for improving model quality. Addressing issues identified during the review and application of PBPK models for human health risk assessments provides an opportunity to improve the science of PBPK modeling as a whole. Attendees will obtain knowledge to improve their models for use in risk assessment, evaluate when and how a model can be applied with a fair degree of certainty, and better understand the constraints and requirements that may otherwise limit use of a PBPK model. Finally, we will conclude with a panel discussion and dialogue on the criteria, processes, methods, and choices that can best assure the use of quality PBPK models in future risk assessments.

W 2689 A PROCESS FOR THE EVALUATION AND IMPLEMENTATION OF PBPK MODELS IN HUMAN HEALTH RISK ASSESSMENTS.

H. A. El-Masri. *NHEERL, U.S. EPA, Research Triangle Park, NC.*

An example process for the review and implementation of PBPK models in human health risk assessments was developed. The process is intended to be rigorous and comprehensive, yet efficient. A collaborative effort between the agency conducting the risk assessment and its contractors is suggested, as well as engaging the original authors of the models being evaluated. Several common errors identified in models being evaluated for use in risk assessments will be discussed, with ways authors can improve the potential for model use. Other models have not been implemented as published for practical reasons that will be described. The ultimate goal of this presentation is to inform and advise authors how to produce models that are more likely to be usable "off-the-shelf" for assessment purposes.

W 2690 APPLICATION-SPECIFIC CONSIDERATIONS IN EVALUATION OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS.

L. M. Sweeney. *TERA, Cincinnati, OH.*

"One size fits all" statements regarding model confidence (i.e., the model is simply "acceptable" or "unacceptable") fail to capture the subtleties inherent in the many ways physiologically based pharmacokinetic (PBPK) models can be used in risk assessment. For example, a model that is acceptable in one context, such as route-to-route extrapolation, many not be adequate for other purposes, such as interspecies extrapolation. Predictions of a given dose metric that may be bounded (e.g., by physiological considerations) could be considered to be reliably estimated by a model for which other dose metrics are not characterized with sufficient confidence. Application-specific considerations should be incorporated in PBPK model evaluation to ensure that these useful tools are appropriately applied in risk assessment.

W 2691 CHARACTERIZATION OF POPULATION DISTRIBUTIONS IN PBPK MODEL PARAMETERS.

H. J. Clewell. *The Hamner Institutes for Health Sciences, Research Triangle Park, NC.*

Population analysis is being applied in PBPK modeling, through rigorous Bayesian statistical analysis and simulation of population variability by Monte-Carlo sampling of parameters. Bayesian analysis in particular provides estimates of population variability and uncertainty in various parameters, but the outcome depends to some extent on the assumed initial (prior) distributions, and can only represent what is known from the study population analyzed, rather than the population as a whole. This talk discusses appropriate choices of parameter distributions such that the full population variability and uncertainty can be captured as well as considerations on how probabilistic modeling results can be integrated into current risk assessment practice.

W 2692 ALTERNATE APPROACHES AND ISSUES IN INTEGRATING PHARMACOKINETIC WITH BENCHMARK-DOSE (BMD) MODELING.

P. M. Schlosser. *NCEA, U.S. EPA, Washington, DC.*

Physiologically-based and traditional pharmacokinetic (PK) models may be integrated with benchmark-dose (BMD) dose-response modeling by using PK-model-predicted internal doses as inputs to BMD analysis, which estimates a point-of-departure (POD) dose equivalent to a no-observed adverse effect level. A human PBPK model would then used to estimate the human-equivalent exposure. However, each time the PK model is revised, which may happen several times during an extensive regulatory review process, the BMD analysis must be revised accordingly; if the analysis is for multiple animal species/genders/target tissues/endpoints, the effort involved can become substantial vs. simply conducting the BMD analysis once using applied exposure levels. Also, if tumors arise in multiple tissues, a single dose metric must be used to correctly estimate total tumor risk with current standard statistical methods. These and other issues to be discussed make the approach for integration of PK and BMD modeling less than straightforward. Different approaches are therefore likely to be preferred for different assessments, depending on the details and level of complexity of the analysis. (Disclaimer: The views expressed in this abstract are those of the author and do not represent the policy of the U.S. Environmental Protection Agency.)

W 2693 EXPLICIT PHARMACOKINETIC MODELING: TOOLS FOR DOCUMENTATION, VERIFICATION, AND PORTABILITY.

J. F. Wambaugh[1], R. Tornero-Velez[2], E. McLanahan[3], Y. Tan[2], W. Setzer[1] and I. Shah[1]. [1]*National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC,* [2]*National Exposure Research Laboratory, U.S. EPA, Research Triangle Park, NC and* [3]*National Center for Environmental Assessment, U.S. EPA, Research Triangle Park, NC .*

Quantitative estimates of tissue dosimetry of environmental chemicals due to multiple exposure pathways require the use of complex mathematical models, such as physiologically-based pharmacokinetic (PBPK) models. The process of translating the abstract mathematics of a PBPK model description into a computational implementation is typically a tedious process fraught with the potential for error. Sometimes key information, such as parameters or even whole equations, is unintentionally omitted from published results. Furthermore, complex models are often interpreted by a wide community of scientists and policy makers; it is important that the language used to communicate the model be explicit and coherent. Fields allied with toxicology, such as systems biology, have created markup languages (i.e. Systems Biology Markup Language) to standardize the syntax of mathematics and documentation, allowing models to be disseminated as supplemental material in articles or through on-line databases. We propose a formal declarative description of PBPK models, PhLexicOn, to assure published models are thoroughly documented and can be readily translated into popular simulation software. Completeness of a model is not sufficient, it must make sense in terms of the relationships among tissue volumes, blood flows and other fundamental biology. Plausibility is assessed though an ontology that defines the appropriate constituents of physiologically-based systems. Tools are in development to: efficiently verify semantic validity based on the ontology, and automate translation into executable simulations to evaluate consistency with published results. PhLexicOn is a framework for documenting, verifying, and translating legacy and state-of-the-art models for re-usability and efficient dosimetry estimation of chemicals for use in human health risk assessments. This abstract does not necessarily reflect U.S. EPA policy.

W 2694 ROLE OF BIOMARKERS IN ASSESSING TOBACCO HARM REDUCTION: A TOXICOLOGICAL PERSPECTIVE.

C. Timchalk and R. Corley. *Pacific Northwest National Laboratory, Richland, WA.*

In 2009, the Family Smoking Prevention and Tobacco Act was passed by Congress, signed into law, and the U.S. FDA was tasked with regulating tobacco. The U.S. FDA's stated goals are to use the best available science to guide the development and implementation of effective public health strategies to reduce the burden of illness and death caused by tobacco products. A potential component of this effort is the implementation of a risk/harm reduction strategy, in which the removal or reduction of harmful ingredients within tobacco products is one possible approach. In this regard, a joint World Health Organization (WHO) and International Agency for Cancer Research (IARC) working group proposed a strategy for lowering toxicant yields in tobacco products based on animal and human toxicity data for individual constituents. This risk based approach requires the prioritization of main stream smoke (MSS) constituents according to their individual risk of developing cancer. In this context, we will explore current and new strategies that have the potential to identify priority constituents for reduction. This will include strategies that utilized conventional approaches to MSS risk assessment which calculate risks according to exposure and hazard. A potential alternative approach for evaluation of MSS constituents exploits a strategy developed by the International Program on Chemical Safety (IPCS) to improve individual chemical risk assessment utilizes a weight-of-evidence approach within a mode-of-action (MOA) framework. Finally, a Quantitative Risk Assessment (QRA) strategy will be considered which combines advanced computational and experimental lung dosimetry, smoking patterns and behavior data, human variability, and available toxicity/cancer potency factors to develop compound-specific comparative estimates of risk for MSS constituents. The primary focus will be placed on both the strengths and weaknesses of the various approaches in the context of a MSS harm reduction strategy.

W 2695 LEGISLATIVE AND REGULATORY OVERVIEW.

M. Zeller. *Pinney Associates, Bethesda, MD.* Sponsor: C. Timchalk.

Tobacco harm reduction policy is a complex and emotionally charged issue. For many decades, the public health community found it difficult to independently evaluate express and implied risk and harm reduction marketing tactics of the tobacco industry. The challenge was exacerbated by the absence of tobacco product regulation. The classic example of the impact of no regulation was the marketing of "light" and "low tar" cigarettes that implied lower risk to smokers. As a consequence, we have witnessed generations of smokers who otherwise may have been motivated to quit that instead compensated for the lower tar and nicotine by increasing consumption, thereby negating any real or perceived reductions in risk. Recent passage of the Family Smoking Prevention and Tobacco Control Act (FSPTCA) changes everything. Under the new law, the U.S. Food and Drug Administration now has a set of regulatory tools to control toxicant delivery, exposure and risk reduction claims, and many other aspects of tobacco product manufacturing and marketing. Similar opportunities for effective, science-based tobacco product regulation are possible under the Framework Convention for Tobacco Control. This presentation will provide an overview of the history of how the tobacco industry made health-related claims in the unregulated marketplace. Key provisions of the FSPTCA will be described, including those which empower FDA

to control toxicant delivery in tobacco products. Critical policy issues will be identified. Research gaps and priorities will also be highlighted. The presentation will also emphasize the relevance of consumer perception and behavior.

W 2696 ASSOCIATION BETWEEN THE DESIGN, CONTENTS, EMISSIONS, USE, AND BIOMARKERS OF EXPOSURE FROM TOBACCO PRODUCTS.

D. L. Ashley. *Center for Tobacco Products, U.S. FDA, Rockville, MD.* Sponsor: C. Timchalk.

Cigarette smoke is a complex mixture of chemical compounds bound to aerosol particles and free in the gas phase. Chemical compounds can be distilled directly from tobacco into smoke or react to form other materials, which are then delivered in the smoke. Published literature reports that many of these chemicals in tobacco smoke are mutagenic, carcinogenic, and addictive. The International Agency for Research on Cancer (IARC) list of carcinogens includes the tobacco-specific nitrosamines, polynuclear aromatic hydrocarbons, volatiles, and heavy metals. Studies have shown that the levels of exposure to these chemicals in smoke can be altered by cigarette design, other chemical additives to the tobacco product and the way people consume the cigarette. In addition, changes to the pH of smokeless tobacco result in higher or lower levels of free nicotine, the form more biologically available. Studies in the laboratory and with smokers indicate that the addition of filter ventilation to cigarettes reduces the levels of smoke delivered using the standard smoking regimen, but this does not adequately reflect the exposure that smokers receive, since they can block the holes, take larger puffs, or take more frequent puffs. Thus, smokers of the same cigarette can inhale a wide variation in the amount of smoke. By examining a wide variety of tobacco products, researchers have determined that the use of specific types of tobacco can alter the relative levels of the chemical constituents in smoke. This can impact the levels of biomarkers in users of these products. By comparing the levels found in the smoke, the characteristics of the tobacco products and how people smoke, we can better understand how these influence the delivery of addictive and toxic chemicals to people. There are critical design and content considerations that can substantially influence the levels of toxic, carcinogenic, and addictive components of tobacco product emissions.

W 2697 TOBACCO CARCINOGEN AND TOXICANT BIOMARKERS: POTENTIAL APPLICATIONS IN TOBACCO HARM REDUCTION AND REGULATION.

S. S. Hecht, J. Yuan and D. K. Hatsukami. *Masonic Cancer Center, University of Minnesota, Minneapolis, MN.* Sponsor: C. Timchalk.

Tobacco products contain complex mixtures of carcinogens and toxicants that are responsible for the deadly effects of these products. Cigarette smoke has over 70 established carcinogens and numerous toxicants. The 20th century method for assessing levels of these substances was machine smoking, but this has severe limitations which are well documented. The 21st century method should integrate tobacco carcinogen and toxicant biomarkers into the assessment of tobacco product carcinogenicity and toxicity. Tobacco carcinogen and toxicant biomarkers are metabolites or protein or DNA adducts of specific carcinogens or toxicants in tobacco products. Highly reliable analytical methods based mainly on mass spectrometry are now available and have been applied in large studies of many of these biomarkers. A panel of tobacco carcinogen and toxicant biomarkers that could be used in studies on tobacco harm reduction and in regulation of tobacco products includes, among others, urinary nicotine and tobacco-specific nitrosamine metabolites, 1-hydroxypyrene, and mercapturic acids of volatile organics. The critical question is: what are the mean biomarker values below which one would see a decrease in tobacco-induced cancer? The answer to this question potentially can be derived from molecular epidemiology studies in which biomarker levels are related to cancer risk. Examples include the relationship of tobacco-specific nitrosamine metabolites to cancer.

W 2698 MODE OF ACTION: ASSESSMENT OF CANCER RISK AND IDENTIFICATION OF KEY DATA NEEDS.

A. R. Boobis. *Centre for Pharmacology & Therapeutics, Imperial College London, London, United Kingdom.*

Cigarette smoking has been causally related to cancer at over a dozen sites in humans, the most commonly affected being the lung. It has proven very difficult to produce an animal model for the lung cancer effects of smoking. Tobacco smoke is a complex mixture of chemicals, both organic and inorganic, many of which have been shown to be mutagenic or carcinogenic in experimental systems. However, inhalation of tobacco smoke also involves exposure to heat and particulates. There is evidence for a number of effects that could be involved in the mode of action for lung cancer. These include mutagenesis by compounds such as nitrosamines and polycyclic aromatic hydrocarbons, cytotoxicity by aldehydes and inflammatory mediators induced by particulates, and the formation of reactive oxygen species. Many biomarkers have been used to study tobacco-related cancer and it important to distinguish exposure from effect. For example, it is still not clear what role is played by some DNA adducts in the etiology of tobacco-related cancer. In order to identify key data gaps, and to identify the most informative biomarkers of effect, the IPCS cancer mode of action framework was used to assess the relationship between smoking and lung cancer. This framework provides a transparent and consistent approach to assessing the mode of action of a chemical carcinogen, the key events involved and their dose-response relationships. A key event is a necessary intermediate process or interaction, though often not sufficient, in cancer causation. The involvement of key events is assessed using criteria based on those described by Bradford Hill, including dose and time dependency, biological plausibility, experimental evidence, consistency and coherence. Through the application of the framework it is possible to identify those areas in which research should be focused to resolve uncertainties.

W 2699 QUANTITATIVE RISK ASSESSMENT: A COMPUTATIONAL DOSIMETRY FRAMEWORK FOR TOBACCO HARM REDUCTION.

C. Timchalk, J. G. Teeguarden and R. A. Corley. *Battelle Pacific Northwest Division, Richland, WA.*

Conventional approaches to evaluate relative risk of mainstream smoke (MSS) constituents have focused on the assessment of exposure and hazard, the latter defined by Reference Concentrations or Cancer Potency Factors. Margin of exposure (MOE)-directed target tissue dosimetry offers an alternative strategy for MSS relative risk prioritization. This strategy exploits advanced computational dosimetry modeling to incorporate smoking patterns and behavior, human variability, and available toxicity/cancer potency. To illustrate the utility of this framework several MSS constituents were evaluated on the basis of their known mode of action (MOA) and key events linking MSS exposure with target tissue dose and response. For example, acetaldehyde is a contact site irritant that causes tumors in the upper respiratory tract of rodents. The MOA involves direct contact irritation and inflammation leading to cytotoxicity as a key initiating event. To enable direct comparison of rat inhalation bioassays with human exposures to MSS, a computational fluid dynamic/physiologically based pharmacokinetic model was developed to compare site-specific doses in each region of the respiratory system. MOEs were calculated by comparing rat nasal tissue and human respiratory tract tissue acetaldehyde concentrations adjusted for exposure duration (Lifetime Average Daily Doses (LADD)). For the rat, steady-state nose-only breathing simulations were conducted at 50 ppm the NOAEL for nasal olfactory degeneration. In humans, oral breathing simulation of a single puff was conducted based on cigarette acetaldehyde yield data. The LADD's in any region of the human respiratory tract ranged from 13- to 50-fold lower than those associated with subchronic acetaldehyde no effect levels in the rat nasal region. This modeling approach can be exploited to compare interspecies regional dosimetry for other important MSS toxicants providing a more quantitative biologically informed approach for prioritizing and targeting MSS constituents and evaluating claims of reduced risk in a tobacco harm reduction strategy.

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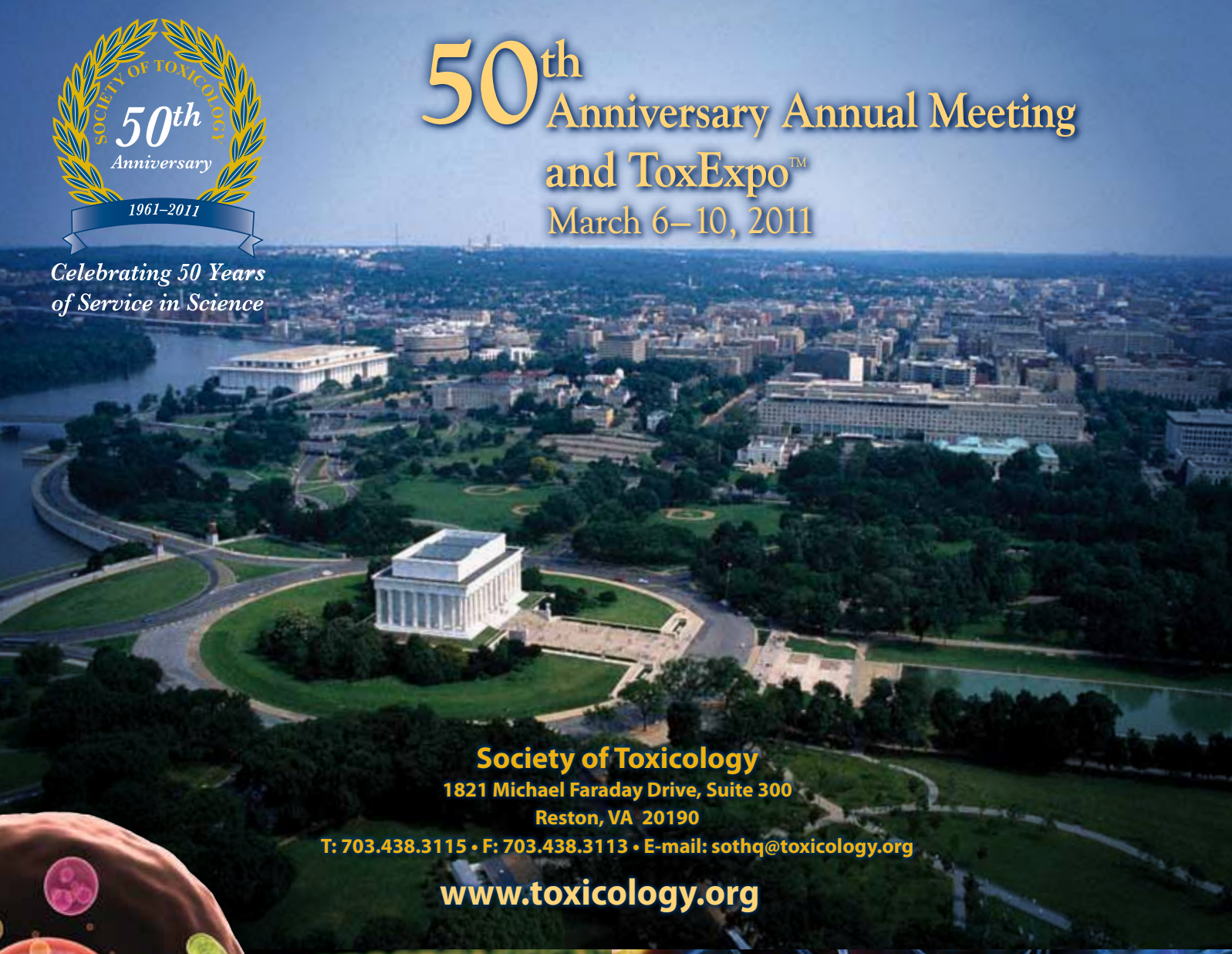

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